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5418

### Production of an Extracutaneous Kidney.\*

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The information gained from observations on the extracutaneous spleen (Barcroft<sup>1</sup>) has been one of the important factors in advancing our knowledge of the physiology of that organ. A careful review of the literature does not reveal a similar method for the study of the kidney. The spleen is peculiarly adapted to such a study because it is so easily mobilized and is composed histologically of considerable muscular tissue. The kidney, however, is available for a similar investigation despite its more highly specialized character and vast structural differences.

The experimental animals in all instances have been female dogs weighing about 10 to 12 kilograms. A very simple procedure to exteriorize the kidney has been employed. An oblique lumbar incision is carried through the skin and subcutaneous tissue to the muscle layer. The muscles are separated by blunt dissection and the kidney fossa exposed. This technique gives sufficient space to lift the intact kidney to the surface of the body where it may be fixed by suturing to the skin edges with silk. Silk has been found to be preferable in this work. In order to prevent self-afflicted

\* This work has been conducted under a grant from the Douglas-Smith Foundation.

<sup>1</sup> Barcroft, J., and Stephens, J. G., *J. Physiol.*, 1927, **64**, 1.

trauma to the exposed organ, it has been found advisable to mould a light plaster cast collar on the animal immediately before operation; postoperative injury incident to the awakening is obviated by suturing a wire meshed basket around the exposed kidney for 24 to 48 hours. A mild inflammatory reaction almost invariably occurs within 48 hours following operation, but it is controlled easily by the usual antiseptic measures. Also it has been found desirable to carefully remove crusts that form on the skin about the exteriorized organ.

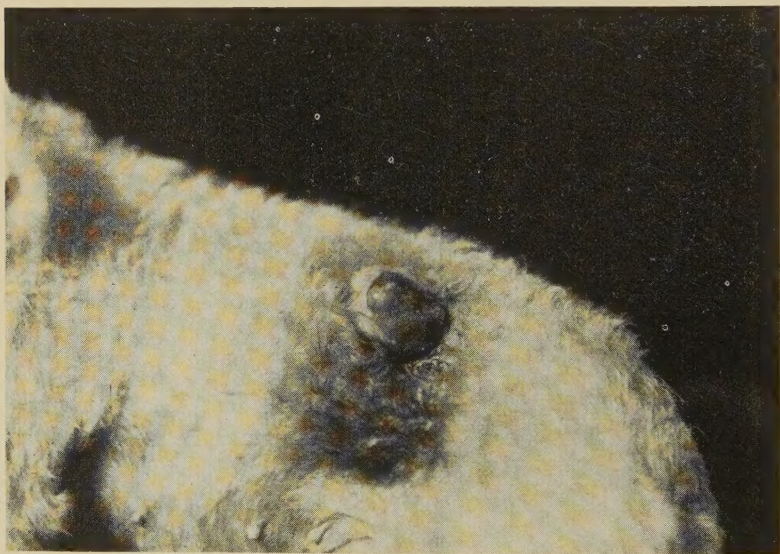


FIG. 1.

Appearance of extracutaneous kidney 5 months postoperative. The epithelial line can be seen distinctly and at present, 8 months postoperative, is completely covered.

A very moderate swelling of the exposed kidney is observed within the first 24 hours after operation, and this increased size persists during the life of the animal. The kidney surface retains its normal color and appearance until it is covered by a thin layer of epithelial tissue that has grown from the healed skin edges.

This procedure has been carried out on a small series of animals (15 dogs) with one kidney exposed and the opposite organ removed and the animals are still living 8 months later with normal kidney function as determined by blood chemistry and intramuscular phenolsulphonephthalein tests.

## Renal Denervation. II. Vascular Changes Induced in the Intact and Denervated Kidney by Rattle Snake Venom.\*

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In a recent report<sup>1</sup> we described a method of antimortem injection to demonstrate in detail the renal vascular bed by roentgenograms and demonstrated the changes seen when the method was applied to the denervated kidney. We will now show the effect of rattle snake venom on the normal and denervated kidneys as demonstrated by the same means. Noguchi<sup>2</sup> described rattle snake venom as a vascular poison resulting pathologically in vascular paralysis and capillary hemorrhages into the tissues. Anuria is a frequent clinical symptom.

We used young dogs throughout. The technique already described<sup>1</sup> was employed in denervating one kidney. After periods exceeding 2 weeks rattle snake venom was injected intravenously. It was found that the minimum lethal dose was above 0.5 of the dry venom per kilo body weight. The kidneys were injected with bismuth oxychloride at intervals up to 48 hours after the administration of the snake venom. The resulting effect varied in degree depending on the dose and the interval elapsing between the injection of the venom and sacrificing of the animal. From the standpoint of the x-ray findings there was profound vascular damage as indicated by beading of the vessels and obliteration of a large portion of the vascular bed varying in degree with the size of the dose. The effect was uniformly more profound in the denervated kidney.

Microscopically the kidney tissue displayed congestion of the capillary tufts, dilatation of the interstitial blood spaces and in a few instances extravasation of blood. Varying degrees of tubular degeneration were noted. The changes seen in the histological section were patchy in distribution with intervening areas of relatively normal tissue. More or less marked endothelial swelling of the arterioles was common. Again the evidences of the action of the venom were more pronounced in the denervated than in the normal kidney.

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\* The present investigation was aided by a grant from the Josiah Macy, Jr., Foundation.

<sup>1</sup> In press.

<sup>2</sup> Noguchi, Carnegie Institute of Washington, 1903, 106-141.

*Conclusions.* It appears that the nervous control of the renal vascular bed acts to protect that organ to some degree at least from certain noxious agents and removal of this nervous control exposes the kidney to greater injury when toxic materials are injected, and allowing them to reach the kidney in marked concentration.

## 5420

## A Biological Reaction for Hyperthyroidism.

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*From the Departments of Surgery and Pathology, University of Illinois College of Medicine.*

A few years ago, Dresel<sup>1</sup> described a new biological reaction for hyperthyroidism based on the observation that the serum of toxic goitre patients when injected into mice rendered their livers glycogen poor. It is upon this reaction that we wish to present this preliminary report. Our observations are in accord with those of Dresel, though we have modified the technique of the test somewhat.

Dresel injects the mice, feeds them oats and water for 48 hours, then starves them for one hour before examining the livers for the presence of glycogen. We have observed, however, that even after several hours of starvation with this method, all the mice may still possess glycogen rich livers, or else the results may vary according to the amount of food which the animals have consumed prior to this starvation period. We, therefore, inject the serum, feed carbohydrates and water for 30 hours, starve the mice 16 hours, inject 1.5 cc. of 10% glucose solution and examine the livers 3 hours later. By this method the amount of food ingested by the mice becomes an unimportant factor, since after this starvation period all the mice have glycogen free livers and each is then supplied with a known quantity of glycogen-forming material. The liver, quickly removed from the animal which has been killed by breaking the neck, is ground with sea sand and 5 cc. of hot water, mixed well with 3 cc. of 20% trichloroacetic acid, centrifuged and filtered. To 20 drops of the filtrate, one drop of Lugol's solution is added. The presence of glycogen is indicated by a mahogany brown color, while a yellow colored solution indicates little or no glycogen.

<sup>1</sup> Dresel, K., and Goldner, M., *Verhandl. d. deutsch. Gesellsch. f. inn. Med.*, 1929, Kong. 41, 534. Dresel, K., *Deutsch. Med Wnschr.*, 1929, **55**, 259.

We have tested the serum of 20 toxic goitre cases, 10 conditions other than thyroid disease, and 2 cases of "thyroid constitution", having experimented with some hundred and seventy-five mice. We have noted that the toxic serum generally renders the liver of the mouse glycogen poor, while the normal serum leaves the liver rich in glycogen. Serum from patients with so-called "hyperthyroid constitution" or possessing the "Basedow stigma" as Dresel terms it, and presenting rapid pulse, sweating and nervousness but normal basal metabolic rates, may give a similar reaction. We are inclined to believe, however, that by the use of smaller amounts of serum such reactions may become fewer. Some of our experiments tend to indicate that iodides diminish the activity of the serum. There also seems to be some indication that the livers of mice injected with toxic serum form glycogen more rapidly than normally, but as stated above, also lose the glycogen more quickly.

Hyperthyroid serum evidently contains some substance which has the power of driving the glycogen from the liver. Whether this is a substance foreign to the normal organism or only increased in amount in the toxic goitre patient we are not at this time prepared to state, though we feel it is probably the latter.

Because he finds that the action of the thyroxin-like acting substance in the serum is 50 to 100 times as effective as the estimated thyroxin per cubic centimeter of blood serum, Dresel believes that some other substance perhaps tyrosin-like enhances the reaction. We have noted that the liver may become glycogen poor many hours sooner than would be expected from thyroxin action, and feel that the reaction may perhaps be dependent upon a substance derived from some other organ of internal secretion, most likely the supra-renal glands. It is even probable that the toxic serum drives the glycogen from the livers not only through increased oxidation but also through altered permeability.

This biological reaction, so simple and yet so delicate, at least offers the possibilities of: (a) a biological test for borderline cases of hyperthyroidism; (b) another method of approaching the study of hyperthyroidism; (c) a reaction as an aid in controlling experimental hyperthyroidism.

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### Adsorption of Physiologically Active Substances by Activated Charcoal.

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(Introduced by F. C. Koch.)

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During the progress of some studies on adsorption, it became necessary to have some information regarding the action of activated charcoal on physiological substances. A search through the literature did not reveal any previous work except a paper by Guerrant and Salmon<sup>1</sup> on the adsorption of quinine. We were not concerned in this case with the mechanism of adsorption or activation. We merely wanted to know whether or not certain drugs would be adsorbed from aqueous solution by activated charcoal. To insure uniformity of results, we decided to use an activated charcoal easily obtainable on the open market.\* The following drugs were studied: strychnine sulphate, brucine sulphate, adrenalin hydrochloride, histamine hydrochloride, acetylcholine hydrobromide, ephedrine hydrochloride, tyramine hydrochloride and diamino butane hydrochloride.

The activity of the drug was studied by intravenous injection. The solutions were prepared as follows: The drug was dissolved in water or physiological salt solution. 25 cc. of the solution were put into a 100 cc. flask as a control solution. Another 25 cc. portion was added to a 100 cc. flask containing 1 gm. of the active charcoal. Both solutions were then shaken for 20 minutes and filtered through a folded filter. The activity of these filtered solutions was determined by intravenous injection. Nine dogs under ether anesthesia were used as the test animals. All injections were made into the left femoral vein.

When strychnine, brucine, adrenalin, histamine and tyramine are treated with activated charcoal they are quantitatively inactivated either through adsorption or modification. In the case of acetylcholine and ephedrine the inactivation is not quite complete.

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<sup>1</sup> Guerrant, N. B., and Salmon, W. D., *J. Biol. Chem.*, 1928, **80**, 67.

\* The charcoal which we used was furnished by the Allied Carbon Company of New York. It is sold under the trade name of "Carboraffin". We are glad to have the opportunity for acknowledging their kindness in supplying us with this material.

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## Mechanism of Secretin Diuresis.

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*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

Owen and Ivy have shown that the intravenous injection of concentrated vasodilatin-free secretin preparations into dogs results in a typical moderate diuresis. Certain characteristics of the diuresis led us to suspect that it was related to, and probably dependent upon the secretory stimulation of pancreatic juice and bile. The following experiments give evidence that this is the case. Following pancreatectomy and ligation of the common bile duct, previously active preparations are without effect on the rate of urine flow. Secretin preparations cause no diuresis if the bile and pancreatic juice resulting from their injection are diverted from the intestine by cannulae. Reintroduction of the collected bile and pancreatic juice into the intestine is followed by the typical diuresis. Diuresis in the intact dog is therefore dependent on the secretion of pancreatic juice and bile, and the entrance of these secretions into the intestine where they may be reabsorbed. It may be noted that in the experiments in which Mellanby<sup>1</sup> reported that secretin had no diuretic effect, the pancreatic juice and bile were simultaneously collected by means of cannulae so that they could not enter the intestine.

5423

## Physiological Effects of Denervating the Carotid Sinus in Dogs.

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The carotid sinus is the seat of reflexes controlling heart rate, blood pressure and respiration. This fact was first demonstrated in acute experiments by H. E. Hering,<sup>1</sup> and has been amply verified

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<sup>1</sup> Mellanby, J., *J. Physiol.*, 1929, **66**, 1.

<sup>1</sup> Hering, H. E., *The Carotid Sinus Reflex on Heart and Blood Vessels*, 1927, Verlag von Theodor Steinkopff (Dresden and Leipzig).

by Heymans,<sup>2</sup> Danielopolu,<sup>3</sup> De Castro,<sup>4</sup> and by one of us (Cromer). The following work was undertaken to study the effects of exercise before and after denervating the carotid sinus to ascertain how vital is the rôle played by the carotid sinus mechanism.

*Method.* Dogs were trained so that heart rate, blood pressure and respiration rate could be determined under basal conditions. After 2½ or three minutes of vigorous exercise, readings were again taken. After a number of control observations had been made over a period of several weeks, the dogs were operated aseptically and the carotid sinus nerve, or Hering's nerve, and the carotid glomerulus and plexus were removed and the carotid sinus region was painted with phenol bilaterally.

Beginning on the day following the operation and thereafter for from 2 to 8 weeks, heart rate, blood pressure and respiration rate were followed before and after exercise.

The accompanying table gives the average results on 5 dogs. The results on the individual dogs compare closely with the results shown in the table.

TABLE I.  
Observations Before and After Denervating Carotid Sinus in Dogs. Average  
5 Dogs.

Heart Rate		Blood Pressure		Respiration		Exercise
Before Exercise	After Exercise	Before Exercise	After Exercise	Before Exercise	After Exercise	
61	122.7	138/79	172/109	18.5	65.5	
	97		149/92		76	5 min. later
	86		144/88		66	10 " "
	73		136/82		59	15 " "
After Operation for Denervation of Carotid Sinuses.						
64.7	134.4	130/82	173/113	17	59	
	102		151/95		44	5 min. later
	91		137/88		53	10 " "
	82		132/83		31	15 " "

The slight variation in the data obtained before and after denervation of sinuses indicates that the physiological rôle of the carotid sinus as a reflexogenic center for controlling blood pressure, heart rate and respiration is readily taken over by other mechanisms in the dogs.

<sup>2</sup> Heymans, C., *The Carotid Sinus and Other Reflexogenic Vaso-sensory Zones*, 1929, London, H. K. Lewis and Co.

<sup>3</sup> Danielopolu, D., and Proca, G., *Arch. Mal. du Coeur*, 1929, 769.

<sup>4</sup> De Castro, F., *Trav. lab. rech. biol. Inst. Cajal*, 1926, **24**, 365; 1928, **25**, 331.

## 5424

## A Comparison of the Buffer Value of Bile and Pancreatic Juice Secreted Simultaneously.

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*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

It is well known that both bile and pancreatic juice play a rôle in neutralizing the acid chyme ejected into the duodenum and that pancreatic juice as a rule is more alkaline (pH 7.8—9.0)<sup>1, 2, 3</sup> than hepatic bile (pH 7.4—8.5)<sup>4, 5, 6</sup> and that gall bladder bile is acid (pH 5.4—6.9)<sup>4, 5</sup> but the titration curves and buffer values of bile and pancreatic juice have not been determined and compared.

In doing this it was thought that more comparable results would be obtained by collecting bile and pancreatic juice from the same animal at the same time. So, under aseptic procedure the gall bladder bile was collected by ligating and removing the gall bladder. The common bile duct was then cannulated, the accessory pancreatic duct tied, and the principal pancreatic duct cannulated; both the cannulae in the common bile duct and the pancreatic duct were connected with sterile rubber balloons large enough to hold a 12-hour sample.<sup>6</sup>

The combined 24-hour collection of the secretions was measured and 10 cc. amounts titrated with N/10 HCl, and the corresponding pH determined with the quinhydrone electrode. This has been done in 5 dogs so far and a typical result is shown in the accompanying chart. In this chart the solid lines represent bile and the dotted lines represent pancreatic juice from the same dog. The lowest line is the gall bladder bile. The other solid lines are hepatic bile and the numbers on them refer to the days after operation that the secretions were obtained. The pancreatic duct cannula became occluded on the sixth day.

It is evident from these curves and our data that hepatic bile may be as alkaline or even more alkaline than pancreatic juice, and that the 2 act in a more or less compensatory manner, *i. e.*, when the

\* Josiah Macy, Jr., Foundation Fellow.

<sup>1</sup> Auerbach and Pick, *Arch. a. d. Reichs-Gesund. am.*, 1912, **43**, 155.

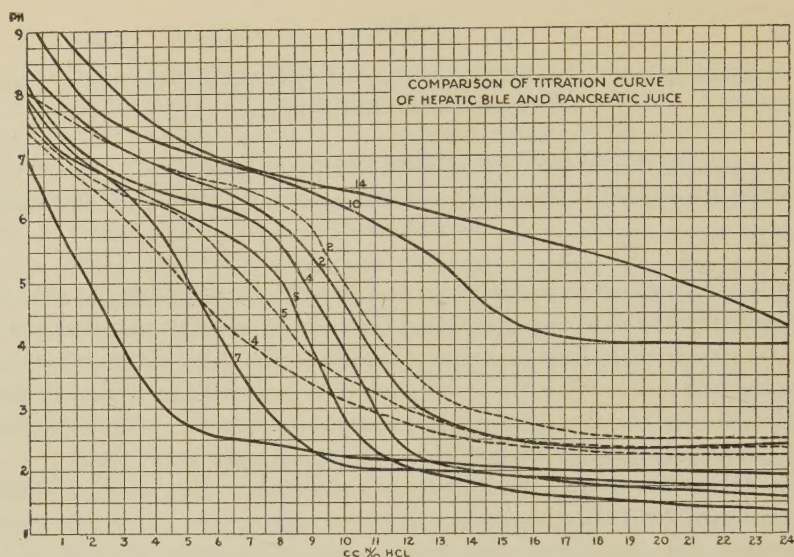
<sup>2</sup> Carnot and Guizewska, *Compt. rend. soc. de biol.*, 1925, **93**, 240.

<sup>3</sup> Farrell and Ivy, *Am. J. Physiol.*, 1926, **78**, 325.

<sup>4</sup> Okada, *J. Physiol.*, 1915, **50**, 114.

<sup>5</sup> Neilson and Meyer, *J. Inf. Dis.*, 1921, **28**, 510.

<sup>6</sup> Drury, McMaster and Rous, *J. Exp. Med.*, 1924, **39**, 403.



buffer action of one goes up, the other tends to go down. The slope of the curves shows a marked similarity and indicates that the buffer action is due to a substance common to both hepatic bile and the pancreatic juice, which substance is shown by other experiments to be sodium bicarbonate.

While the pancreatic juice is flowing, the hepatic bile is quite constant and in 10 instances lies very close to the curves for second and fourth day. This is shown in the chart. When, however, the pancreatic juice is blocked, the hepatic bile may vary quite widely in buffer action as indicated on the chart, usually increasing markedly in buffering action in an attempt to carry to the intestine the alkali previously carried by the pancreatic juice. The amount of pancreatic juice secreted is as a rule more than the amount of bile secreted, when both secretions are flowing freely, so that the total buffer secreted by the pancreas is always greater than the total buffer secreted by the liver under the conditions of our experiment.

5425

### Demonstration of a Dog Maintained for Sixteen Weeks Solely by Jejunal Alimentation in the Presence of Loss of Gastric Juice.

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*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

In order to answer certain questions relative to the general problems of gastric secretion and intestinal obstruction, some method of maintaining dogs indefinitely by jejunal alimentation was desired. Also, the clinician is not infrequently confronted with the question of the proper method and pabulum to use in certain jejunostomy cases. The difficulty that is generally encountered in attempting to feed animals and man via a jejunostomy is that diarrhea and enteritis are so likely to occur.

Obviously, such a method should consist, first, of a bland, non-irritating, easily digested pabulum containing all the essential food elements and, second, it should be administered slowly, simulating the manner of emptying of the stomach. It might be thought that the pabulum should be predigested with gastric juice and pancreatin and glucose added. We have tried this a number of times, but have found that such a mixture was irritating to the bowel.

After considerable preliminary experimentation on jejunal fistula dogs, a pabulum was found by one of us (H. G. Scott) which was bland and non-irritating. It consists of the following elements: water, 3000 cc.; whole milk, 3000 cc.; flour, 300 gm.; cane sugar, 150 gm.; peptone, 100 gm. The mixture is cooked, giving attention to certain details which will be given in a later, more complete paper (pH 6.0). Four hundred cc. of the mixture is fed 3 to 5 times in 24 hours diluted with 200 cc. of tap water.

When the jejunal feedings are first started the intestine will not tolerate 600 cc., but smaller quantities must be given more frequently. To each feeding 1 gm. of pancreatin is added. From 6 to 10 gm. of salt are added per day to maintain a normal level of blood chlorides. To the various feedings are added daily, but not to every feeding, 10 cc. cod liver oil emulsified in bile (if not emulsified in bile it is likely to cause diarrhea), 2 drops viosterol, one egg-yolk, "yeast foam" and carotin. The mixture is introduced by gravity into the fistula at a slow rate of from 2 to 5 cc. per minute. If introduced at a more rapid rate, nausea and vomiting are likely to be

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\* Josiah Macy, Jr., Foundation Fellow.

initiated. When introduced properly the dog uniformly falls to sleep.

The dog demonstrated has a pouch of the entire stomach with vagi intact and a jejunal fistula. The pylorus was cut across, the duodenal end closed and the gastric end brought to the outside. The jejunal fistula was made prior to this operation. This animal has been fed solely through the jejunal fistula for 18 weeks and has been losing all gastric juice (400-600 cc. daily) for a period of 16 weeks. He has received nothing subcutaneously or intravenously except  $1\frac{1}{2}$  gr. ferric citrate subcutaneously during the past 3 weeks every other day because he became somewhat anemic. His blood is now normal. The blood chloride level can be varied at will by removing or adding sodium chloride to his diet. The dog now weighs as much as he did prior to operation (24 pounds). At  $2\frac{1}{2}$  months a stomatitis with ulcers appeared, which disappeared as vitamins were added to the diet and the mouth was washed daily with lemon juice.

## 5426

### Studies on the Specificity and Behavior of Blood and Tissue Lipases.

LATHAN A. CRANDALL, JR., AND IAN S. CHERRY. (Introduced by A. C. Ivy.)  
*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

It has long been known that the lipase normally present in the blood will not split true fats,<sup>1</sup> and that the pancreas contains much greater amounts of a true fat-splitting lipase than do any other organs. A search of the literature has failed to reveal any report of the appearance in the blood of a fat-splitting lipase in experimental pancreatic injury, although it is well known that such injury increases the blood diastase.

We have studied the blood lipases of 5 dogs with experimental pancreatic injury; in one of these animals, the pancreatic ducts were ligated, in one the tail of the pancreas was doubly ligated and severed from the body, and in the remaining 3 the body of the pancreas was removed, leaving the head and tail free in the mesentery. The blood lipases were followed in these animals, using olive oil and ethyl butyrate as substrates. In making determinations on olive

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<sup>1</sup> Oppenheimer, C., *Die Fermente und ihre Wirkungen*, Leipzig, 1925, 5th ed., 489.

oil a 50% emulsion of the oil previously freed from fatty acid and containing 5% of acacia as an emulsifying agent and 0.2% of sodium benzoate as a preservative was used. One cc. of serum, 2 cc. of the olive oil emulsion, 3 cc. of distilled water, and 0.5 cc. of a normal phosphate buffer adjusted to pH 7.0 were shaken together and incubated at 40°C. for 24 hours. For ethyl butyrate determinations, 1 cc. of ethyl butyrate (absolute) was substituted for the olive oil emulsion. After incubation 3 cc. of 95% alcohol and 3 drops of 1% phenolphthalein were added and the mixture titrated with N/20 acid to a faint pink. Controls containing 1 cc. of serum inactivated by heat were run simultaneously. A lipase capable of hydrolyzing olive oil appeared in the blood of all animals very promptly, the highest values observed being attained within 24 hours. In the animal with ligation of the pancreatic ducts this lipase had completely disappeared within 20 days and in the animal with separation of the tail within 14 days. Of the 3 animals with removal of the body of the pancreas 2 are still living, 50 and 30 days after operation, and an olive oil lipase is present in the blood of both, although it has decreased steadily since operation. The esterase which hydrolyzes ethyl butyrate was not consistently changed; in the animal with duct ligation it first fell slightly, then as the olive oil splitting lipase disappeared, it rose somewhat above the control value. In one of the animals with removal of the body of the pancreas it rose suddenly to 3 times control value on the 30th day and has remained above normal since that time.

The blood lipases have been studied in 7 dogs with Eck fistulas, 51 determinations having been made on these animals. In 31 of these determinations positive values for an olive oil splitting lipase have been obtained. This lipase usually does not appear until 2 or 3 weeks after operation, and the longer the period since operation the more commonly the olive oil lipase is present. Even in the older animals, however, it is subject to marked variation which may be related to alimentation, since this lipase usually disappears on fasting. The highest values obtained for olive oil lipase in the Eck fistula animals have been about one-third of the values found in the dogs with pancreatic injury. For the most part the serum esterase (ethyl butyrate) determinations in Eck fistula dogs have yielded values well within normal limits, although we have occasionally found high values; similar findings for ethyl butyrate esterase have been reported by Whipple.<sup>2</sup>

Studies have been made on 5 dogs with complete obstructive

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<sup>2</sup> Whipple, G. H., *Bull. Johns Hopkins Hosp.*, 1913, **24**, 357.

jaundice. In all of these relatively large amounts of an olive oil splitting lipase have appeared in the blood, while the ethyl butyrate splitting esterase has not changed. The olive oil lipase is present about 6 hours after the operation and reaches its maximum in about 48 hours. However, in one animal which refused food postoperatively no such lipase appeared in the blood until the 72nd hour.

Determinations of the lipases acting on olive oil and on ethyl butyrate have been made on glycerol extracts of brain, muscle, spleen, kidney, liver, intestinal mucosa, lung and pancreas. All of these extracts were active on ethyl butyrate, liver extract being by far the most potent. Pancreas, intestinal mucosa, liver, and spleen extracts were found to contain a lipase capable of splitting olive oil, activity being in the order named. A trace of olive oil lipase was present in the kidney extract.

The appearance in the blood of dogs with pancreatic injury of a lipolytic activity which is not normally present is best interpreted, we believe, as a demonstration of the specificity of pancreatic lipase. It could also be due to the appearance in the blood of a coenzyme which activates the normal blood lipase. The lipolysis of olive oil by serum after liver injury is more difficult of interpretation. It could be due to absorption of this lipase from the injured liver into the blood. The possibility that this lipase is normally passing into the blood from the gastro-intestinal tract and that the injured liver fails to remove it while the normal liver acts as a barrier to this substance must also be considered. We consider the latter supposition the more likely; further work will be done on this question.

## 5427

**Presence of an Olive Oil Splitting Lipase in the Blood of Patients With Multiple Sclerosis.**

LATHAN A. CRANDALL, JR., AND IAN S. CHERRY. (Introduced by A. C. Ivy.)

*From the Department of Physiology and Pharmacology, Northwestern University Medical School.*

Recently Brickner<sup>1</sup> has reported that the blood of patients with multiple sclerosis causes a myelinolysis of the spinal cords of rats *in vitro*; he has also found that serum from these patients becomes less alkaline on standing than does serum from normal individuals;<sup>2</sup>

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<sup>1</sup> Brickner, R. M., *Arch. Neurol. and Psychiat.*, 1930, **23**, 715.

<sup>2</sup> Brickner, R. M., *Bull. of the Neurol. Inst. of N. Y.*, 1931, **1**, 105.

this difference is accentuated by the presence of lecithin. These changes he ascribes to the presence of increased amounts of a lipolytic substance in the blood of patients with multiple sclerosis. He believes that this substance may be an etiological agent.

We have made studies of the serum lipases of 19 patients with multiple sclerosis, using as substrates olive oil and ethyl butyrate according to the technique previously described by us.<sup>3</sup> This is a titration method, employing N/20 NaOH and phenolphthalein. The error is such that we only consider values above 0.2 cc. as positive. In the cases studied we have found values of 0.3 cc. or above, using olive oil as a substrate, in 12 or 63%.

Sera from 146 dispensary patients have been used as controls. Of these sera 140 showed no trace of olive oil lipase, 6 gave values of 0.3 cc. or above. On these 6 false positives the clinical diagnoses were astigmatism, cataract, hypertension, arthritis, cerebellar medulloblastoma, and traumatic psychosis. Thus 95.9% of the controls gave negative results for olive oil lipase.

In view of the finding of an olive oil splitting lipase in the blood of animals with experimental liver or pancreatic injury a special study was made of cases with a clinical diagnosis of liver or pancreatic involvement. Eleven such cases were studied. Seven, or 63% of them, showed the presence of an olive oil splitting lipase in the blood serum. The diagnoses on the cases showing positive results were: carcinoma of the biliary tract, carcinoma of head of pancreas (2 cases), stone in common duct, and cirrhosis (3 cases). The diagnoses on cases of liver involvement with negative serum lipase were: gall stones with slight icterus, cirrhosis, catarrhal jaundice, and metastatic liver. Thus it appears that an olive oil splitting enzyme is present in most cases of diffuse involvement of the liver parenchyma.

No variation from normal values for ethyl butyrate esterase has been observed, except that in some cases of severe and long-standing liver disease with cachexia (especially cirrhosis) the esterase titre is low.

This abnormal serum lipase which appears in multiple sclerosis, in experimental hepatic and pancreatic damage, and in clinical cases of hepatic disease, may possibly be an etiologic factor as suggested by Brickner. It appears more likely, however, that it is merely an evidence of pathology in the liver or pancreas.

We wish to express our appreciation to Dr. Pollock and other

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<sup>3</sup> Crandall, L. A., Jr., and Cherry, I. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 572.

members of the Department of Neurology, Northwestern University Medical School, and to Dr. Minke of the Oak Forest Infirmary, for cooperation which made these studies possible.

## 5428

## Staining Differences of Nuclei in Hepatic Cells.

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*From the Department of Anatomy, Northwestern University Medical School.*

During a course of experiments with fixatives we observed an apparent differential staining of nuclei in liver cells. This was obtained after fixation of the tissue in a solution consisting of:

Potassium bichromate	2 gm.	Sulphuric acid (conc.)	1.75 cc.
Sodium sulphate	0.75 "	Acetic acid (conc.)	5 "
Mercuric sulphate	3.5 "	Distilled water	87 "

Liver tissue from the rabbit, cat, and dog was fixed for about 20 hours, after which it was dehydrated, imbedded in paraffin, and cut into  $10\mu$  and  $5\mu$  sections. Some sections were stained with Mallory's triple connective tissue stain while others were stained in hematoxylin and eosin.\*

*Observations.* 1. Some nuclei of mononuclear liver cells appear blue, while others appear red. 2. Both nuclei in some binucleate liver cells appear blue, while in other cells both appear red. 3. In some binucleate liver cells one nucleus appears blue, the other red. 4. In the blue staining nuclei the nucleoli, which may be two in number, stain blue. 5. In the red staining nuclei, the nucleoli are as a rule red, but additional blue nucleoli may be present. 6. The nuclear ground-substance (enchylema) and the nuclear sponge-work stain red in the red nuclei and blue in the blue ones. 7. No apparent difference in the staining affinity of the cytoplasm of the cell body proper has been observed.

Schäfer,<sup>1</sup> Jordan,<sup>2</sup> and Bloom<sup>3</sup> do not describe any differences in

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\* Blue indicates affinity for aniline blue in Mallory's stain or hematoxylin in the hematoxylin and eosin stain; red indicates affinity for acid-fuchsin in Mallory's stain or eosin in the hematoxylin-eosin stain.

<sup>1</sup> Schäfer, E. A., *Quain's Anatomy*, 1912, **2**, 1. Longman, Green and Co., London.

<sup>2</sup> Jordan, E. H., *A Text-book of Histology*, 1930. W. B. Saunders Co., Philadelphia.

<sup>3</sup> Bloom, W., In Maximow and Bloom, *A Text-book of Histology*, 1930. W. B. Saunders Co., Philadelphia.

the staining of nuclei in liver cells. The latter author comments that in view of the numerous functions which the liver cells perform it is quite striking that there should be such a marked similarity in appearance in all the liver cells. Wilson<sup>4</sup> states regarding nuclei in general: "The nuclear framework undergoes great changes of staining-capacity in different phases of the cell-cycle and may even completely lose its affinity for the basic dyes, becoming purely oxyphilic like linin or general cytoplasm." He suggests further that the differences in the affinity of the nuclear framework may be interpreted as . . . "but passing phases, more or less marked and enduring, of one fundamental substance." A somewhat similar explanation is offered by him in regard to nucleolar affinities. This may lead to the view that the differences in the nuclear affinities observed in the liver cells are due either to their varying physiological state in life or to the particular fixative employed.

## 5429

**Determination of the Amount of Blood in the Central Nervous System Following Injections of Hypertonic Solutions.**

ARTHUR WEIL. (Introduced by S. W. Ranson.)

*From the Institute of Neurology, Northwestern University Medical School.*

The theory that the volume of blood and the volume of fluid within the cranium are inversely related to each other (Monro-Kellie-Burrows) has never been subjected to the test of the direct determination of the amount of blood in the central nervous system following dehydration after injections of hypertonic solutions. Wolff and Forbes<sup>1</sup> noticed contraction of the pial blood-vessels under such experimental conditions, while contrary to their observation Kubie and Hetler<sup>2</sup> described an increase in the color intensity of the brain cortex in color photographs taken before and after injections of 20-30% sodium chloride solutions.

Based on the observations of Voigt<sup>3</sup> that blood, brain and spinal cord do not retain colloidal silver but are free of silver 4 hours after the injection of collargol, the following method was applied for the

<sup>4</sup> Wilson, E. B., *The Cell in Development and Heredity*, 1925, 85. The Macmillan Company, New York.

<sup>1</sup> Wolff, H. G., and Forbes, H. S., *Arch. Neurol. and Psych.*, 1928, **20**, 73, 1035.

<sup>2</sup> Kubie, L. S., and Hetler, D. M., *Arch. Neurol. and Psych.*, 1928, **20**, 749.

<sup>3</sup> Voigt, J., *Biochem. Z.*, 1914, **63**, 409.

determination of the amount of blood in the central nervous system: Collargol (Heyden) was dissolved in distilled water, corn-syrup added as a protective colloid and the solution added to an equal volume of either 1.7% or 30% solution of sodium chloride or 100% solution of anhydrous dextrose. The collargol solutions in 0.85% or 15% sodium chloride or 50% dextrose respectively were injected intravenously into dogs under ether anesthesia, 8 cc. per kg. weight. Five to 10 minutes following the injection approximately 30 cc. of blood was taken from the femoral artery and shaken with a few milligrams of heparin to prevent clotting. Immediately thereafter the dogs were killed by puncture of the medulla oblongata. Both jugular veins, carotid arteries and the medulla oblongata *en masse* were ligated and brain and spinal cord removed. After drying at 120° for the determination of water, blood, brain and spinal cord were ashed with the Neumann method and the silver was determined volumetrically with the Vollhard method, m/50 solutions being used. The amount of blood in brain and spinal cord was calculated from the amount of silver found as demonstrated in the following example: Dog 13.6 kg., injected 108 cc. of 4% collargol in 0.85% sodium chloride.

30 cc. of blood used 17.25 cc. m/50 ammonium sulfocyanate:

78.8 gm. brain   "   2.90   "   "   "   "   "   = 5.04 cc. blood.  
14.5 gm. spin. cord   "   0.25   "   "   "   "   "   = 0.43 cc. blood.

Table I will give the averages of the experiments:

TABLE I.

Injected	Weight			Amount of blood				% of Water		
				Brain		Spinal cord				
	Body kg.	Brain gm.	Spinal cord gm.	cc.	per 100 gm.	cc.	per 100 gm.	Brain	Spinal cord	Blood
0.85% NaCl 9 dogs	11.4	66.1	13.2	5.5	8.3	.91	6.9	77.2	67.8	78.4
15% NaCl 5 dogs	12.1	66.3	14.9	8.8	13.2	3.7	24.8	76.8	67.0	78.8
50% dextrose 5 dogs	10.5	75.6	13.3	5.3	7.0	1.9	14.3	77.7	67.1	78.4

It may be concluded that the dehydrating effect of the 15% sodium chloride solutions was more pronounced than that of the 50% dextrose, and that the amount of blood following dehydration after injection of 15% salt solutions was increased from 8.3 cc. per 100 gm. brain in the controls to 13.2 cc. in the dehydrated brains. Based on Ranke's figures of a total amount of blood equal to 6.6%

of the body weight, brain and spinal cord of dogs (without meninges) under ether anesthesia contain after injection of 0.85% salt solutions 0.9% of the total blood, after injection of 15% salt solution 1.6%, and after injection of 50% dextrose 1.1% of the total amount of blood.

## 5430

### A Method for Accurately Locating Points in the Interior of the Brain.

S. W. RANSON AND W. R. INGRAM.

*From the Institute of Neurology, Northwestern University Medical School.*

With the instrument devised by Horsley and Clark<sup>1</sup> it is possible to stimulate or destroy any desired point in the interior of the brain. When properly adjusted to the head of the animal by grasping devices inserted in the external auditory openings and the orbits, the base line of the instrument passes through the center of the external auditory meatus and through the lower border of the orbit. The zero horizontal plane is parallel to the base line but above it one-third of the distance from the interaural line to the vertex. The zero point is at the intersection of this plane and the midsagittal plane with one at right angles to them passing through the interaural line. The instrument is so constructed that it is possible to place the tip of a fine electrode at any desired position with reference to this zero point, for example, at a point 7 mm. to the right, 4 mm. rostral and 2 mm. dorsal to the zero point.

In order to use the instrument it is necessary to know the location of the point which is to be stimulated or to be destroyed by electrolysis, with reference to the rectilinear coordinates of the instrument. For this purpose we have prepared cats' brains in the following manner:

With the instrument in position on the head the cat was injected with 10% formalin. After the brain was thoroughly hardened *in situ* the skull was removed in certain small restricted areas. Through these openings perfectly straight 18 gauge copper wires were inserted with the aid of the instrument. Three wires were inserted horizontally from behind through the cerebellum and tentorial notch to the rostral extremity of the brain. These were

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<sup>1</sup> Horsley, V., and Clark, R. H., *Brain*, 1908, **31**, 45.

located in the zero horizontal plane and while one was in the mid-line the other 2 were 5 mm. on either side of the midline. Four other wires were inserted vertically in a plane 17.5 mm. in front and 2 more in a plane 7.5 mm. in front of the zero point.

The brain was then dissected out and imbedded in celloidin. The horizontal wires were withdrawn, leaving minute tunnels through the brain and celloidin in the zero horizontal plane. The 2 wires in the frontal plane located 7.5 mm. rostrally were also pulled out, leaving similar tunnels marking the position of that plane. The block was then cut along the rostral surface of the 4 wires in the frontal plane 17.5 mm. in front of the zero point and the brain cut with the microtome into serial sections parallel to the frontal plane thus established.

Each frontal section has in it 3 small round holes produced by the wires inserted on the zero horizontal plane. One centimeter divided by the number of sections included between the 17.5 and 7.5 mm. planes gives the thickness of each section in terms of the unimbedded brain. This eliminates the error which would be introduced by shrinkage during imbedding and makes it possible to assign each section to its correct frontal plane.

Since the frontal plane to which each section belongs is accurately known and since each section contains 3 small round holes in the zero horizontal plane from which measurements can be made it is possible to fix with great accuracy the location of any nucleus or fiber tract and to express it in terms of rectilinear coordinates with reference to the zero point.

## 5431

### Carbohydrate Metabolism in Degenerated Striated Muscle.

DORA K. FISHBACK AND HAMILTON R. FISHBACK.

(Introduced by J. P. Simonds.)

*From the Department of Pathology, Northwestern University Medical School.*

Twenty-three rabbits used in these experiments were put under light ether anesthesia, and the gastrocnemius muscle injured either by freezing with carbon dioxide snow or by multiple light blows. After 24 to 72 hours the animals were given amytal intraperitoneally and the gastrocnemii removed after being frozen solidly with

carbon dioxide snow and ethyl chloride.<sup>1</sup> The muscles were analyzed quantitatively for glycogen,<sup>2</sup> lactic acid,<sup>3, 4</sup> and organic and inorganic phosphorus.<sup>5</sup>

The average glycogen value in normal rabbit gastrocnemii was 603 mg. per 100 gm. of muscle. This dropped to 258 mg. in those muscles injured by previous freezing, and to 103 mg. in the contused muscles. From microscopic studies of the lesions it was evident that degenerative changes were more profound in the contused than in the frozen muscles. With this decrease of glycogen there was a concomitant increase of lactic acid from a control average of 19 mg. per 100 gm. of muscle, to 53 mg. in the frozen muscles and 66 mg. in contused muscles. Phosphocreatine decreased markedly from an average control value of 66.3 mg. per 100 gm. to 7 mg. in the contused muscles. Contrary, however, to expectations the inorganic phosphorus likewise decreased slightly, from 44.5 to 36.1 mg. per 100 gm.

Microscopically there was extensive molecular degeneration of the muscles, with consequent interference with the capillary blood circulation, although the larger vessels had free circulation.

The results given suggest damage to the glycogenic function or to the glycogen storing capacity of the muscles. The still-active glycogenase depleted the muscle glycogen store, with the subsequent formation of lactic acid. While some of the acid may have diffused out into the surrounding tissues or larger vessels, enough remained to show the character of the change. This increase of lactic acid was accompanied as usual by a marked drop of phosphocreatine. The decrease of inorganic phosphorus also may indicate loss by diffusion.

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<sup>1</sup> Davenport, H. A., and Davenport, H. K., *J. Biol. Chem.*, 1928, **76**, 651.

<sup>2</sup> Pflüger, E., *Arch. f. die Ges. Physiol.*, 1909, **129**, 362.

<sup>3</sup> Friedemann, T. E., Cotonio, M., Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

<sup>4</sup> Davenport, H. A., and Cotonio, M., *J. Biol. Chem.*, 1927, **73**, 359.

<sup>5</sup> Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1929, **81**, 629.

5432

### Morphological Changes Induced in the Liver by Acute Passive Congestion.

J. W. CALLAWAY. (Introduced by J. P. Simonds.)

*From the Department of Pathology, Northwestern University Medical School.*

This is a continuation of the experimental work on the liver of the dog begun by Doctor Simonds and Doctor Brandes.<sup>1, 2, 3, 4, 5</sup>

The morphological changes induced in the liver by acute passive congestion lasting from 10 to 30 minutes, are being studied. The slides are taken from the same animals used in the above experiments with some additional ones from dogs in which a detailed study of blood and lymph changes was not made.

The method of producing acute passive congestion has been described in the preceding papers. Briefly, it consists in producing mechanical constriction of the hepatic veins between the liver and the diaphragm by clamping with an intestinal clamp or by placing rubber tubing about these veins, anterior to and excluding the inferior vena cava. With the veins constricted, the liver can be seen to enlarge and darken, and the veins in the mesentery can be seen to dilate while the intestine assumes a bluish color.

After 10 to 30 minutes, the constriction is released, and the laparotomy wound is closed. All the work is done aseptically under ether anesthesia.

The dogs are then killed at intervals of 12 to 72 hours with ether. Some of the last dogs have been allowed to live 1 week. After autopsy, sections from several lobes of the liver are prepared routinely.

In studying these slides, an attempt is being made to estimate the amount of damage done. This is being done by comparing the area of the tissue which seems little if at all damaged, with the total area of the portion of the section examined microscopically. From 15 to 20 low power fields—measuring 1.3 mm. in diameter, or 1.327,326 sq. mm.—are studied per slide. The area of the normal tissue is computed arithmetically from measurements with a micrometer which fits in the eyepiece. The portions of the fields to be measured are

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<sup>1</sup> Simonds, J. P., *J. Immunol.*, 1927, **13**, 11.

<sup>2</sup> Simonds, J. P., and Brandes, W. W., *Am. J. Physiol.*, 1925, **75**, 201.

<sup>3</sup> Simonds, J. P., and Brandes, W. W., *Am. J. Physiol.*, 1925, **75**, 320.

<sup>4</sup> Simonds, J. P., and Brandes, W. W., *J. Immunol.*, 1927, **13**, 1.

<sup>5</sup> Simonds, J. P., and Brandes, W. W., *Am. J. Physiol.*, 1928, **80**, 623.

split into geometric figures whose areas can be obtained by multiplication of their heights and widths according to the rules of arithmetic.

*Results.* The morphological changes induced may be summarized as follows: 1. Dilatation of the central veins and neighboring capillaries. 2. Engorgement of the central veins and neighboring capillaries with r.b.c. and granular eosinophilic debris. 3. Formation of conglutination thrombi in the central veins. 4. Areas of focal necrosis about the thrombosed central veins. 5. In some cases, a generalized, but slight, infiltration of the tissue with polymorphs. 6. Degenerative changes in the cells about the central veins, a. narrowing of cells; b. loss of characteristic cord-like structures, piling up of cells with ill-defined borders and fragmentation of cells; c. loss of staining affinity, both by nucleus and by cytoplasm; d. increased and coarse granular appearance of cytoplasm; e. vacuolization of cytoplasm; f. hemosiderm pigmentation; g. decrease in number of nuclei; h. degenerative changes in nuclei, pyknosis, karyolysis. 7. Hyperplasia of portal canals. 8. Edema of portal canals. 9. Slight degenerative changes in cells surrounding the portal canals. 10. In those slides studied for quantitative changes, as much as 90% to 94% of the tissue has been damaged.

The production of areas of focal necrosis is particularly interesting, for this apparently bears out Mallory's<sup>6</sup> hypothesis as to the origin of focal necrosis in the liver in typhoid. That focal necrosis is caused by the plugging of capillaries by enlarged Von Küpfer cells, or the proliferation of endothelium, has been doubted by later workers.

### 5433

#### Cinchophen Poisoning.\*

T. P. CHURCHILL AND F. H. VAN WAGONER. (Introduced by J. P. Simonds.)

*From the Department of Pathology, Northwestern University Medical School.*

Cinchophen is usually given in therapeutic doses of  $7\frac{1}{2}$  grains 3 times a day. Considering 150 pounds as the normal or average weight, this is a dose of 22 mg. per kilo.

In order to produce cinchophen poisoning quickly 3 dogs were

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<sup>6</sup> Mallory, F. B., *J. Exp. Med.*, 1898, **3**, 611.

\* This work was aided by a grant from the American Medical Association. We wish to thank Dr. H. R. Fishback for donating the dogs with kidney damage.

given 27 times the therapeutic dose, *i. e.*, 595 mg. per kilo mixed with their food. Every 3 or 4 days the blood sugar, urea nitrogen and Van den Bergh were determined. In this group the dogs refused to eat after from 8 to 10 doses of the drug. The urea nitrogen showed a marked rise, then a depression followed by death. Dog 1 lived 10 days, Dog 4, 17 days, and Dog 2, 20 days. At autopsy these dogs showed from one to 3 acute gastric ulcers and a number of yellowish areas over the surface of the liver. On microscopic examination the liver showed varying degrees of liver necrosis from small areas of coagulation necrosis just beneath the capsule, as in Dog 1, to complete disappearance of liver cells in small areas, as in Dog 4.

Dogs 5 and 6 were given the same dose of cinchophen per kilo. The blood sugar, blood urea nitrogen and bromsulphalein retention determinations were made. Dog 6 ate 2 doses, refused to eat more, and died 9 days later. The urea nitrogen increased at first, then decreased in amount. When the urea nitrogen decreased the bromsulphalein test showed increased retention of the dye. Dog 5 ate 3 doses of the drug, then refused to eat. The changes in blood determinations were similar in that the urea nitrogen was slightly increased in amount, then decreased, while the bromsulphalein was retained. After fasting the bromsulphalein retention subsided and the urea nitrogen increased in amount. At this time the dog ate another dose of the medicine and a more marked retention of bromsulphalein resulted. The urea nitrogen was again decreased in amount. After the retention had become less the same results were seen after another 2 doses given by stomach pump. The autopsy on Dog 5 revealed a diverticulum of the duodenum and yellowish areas over the liver. Dog 6 showed no gastric ulcers but showed slightly opaque areas over the liver surface. Microscopic examination revealed areas of coagulation necrosis.

In another 2 dogs kidney damage was produced by a method of clamping the renal artery devised by Dr. Ivy and his coworkers.<sup>1</sup> After recovery (11 months later) these dogs were fed the normal dose of cinchophen, *i. e.*, 22 mg. per kilo of weight. The same determinations were made as in the preceding series. Dog 8 serves as a good example for these 2. The urea nitrogen shows a slight rise, then a slight decrease as the bromsulphalein retention is increased. The bromsulphalein shows an increasing retention with fluctuation in degree until two feedings were missed and a drop occurs. These dogs are still living.

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<sup>1</sup> McEnery, Meyer and Ivy, *J. Lab. and Clin. Med.*, 1927, **12**, 349.

5434

### Effect of Mechanical Obstruction of the Hepatic Veins upon Guanidine Content of the Blood.

OPAL E. HEPLER AND J. P. SIMONDS.

*From the Department of Pathology, Northwestern University Medical School.*

Increase in the guanidine content of the blood has been reported in tetany following parathyroidectomy (Burns and Sharpe,<sup>1</sup> Koch,<sup>2</sup> and Paton and Findley<sup>3</sup>); in hypertension (Major and Weber<sup>4</sup>); in Laennec's cirrhosis of the liver and in Banti's disease (Ellsworth<sup>5</sup>); in eclampsia (Minot and Cutler<sup>6</sup>); and in poisoning with carbon tetrachloride and chloroform (Minot and Cutler<sup>7</sup>). In the last 3 of the above conditions there is an acute severe damage to the liver. Injury to the liver can be induced by mechanical obstruction of the hepatic veins. It appeared to be worth while, therefore, to compare the effects of injury to the liver induced by this method on the guanidine content of the blood with those obtained by Minot and Cutler in cases of liver injury caused by acute poisoning and eclampsia.

Under complete ether anesthesia and with rigid aseptic precautions we mechanically obstructed the hepatic veins by the method described by Simonds and Brandes.<sup>8</sup> Samples of blood were taken before the operation and 24, 48 and, in some instances, 72 hours after the operation. Guanidine was determined by the method described by Major and Weber.<sup>9</sup> The average of our normal determinations was 0.347 mg. of guanidine per 100 cc. of blood, with a maximum of 0.417 mg. and minimum of 0.303 mg. The figures given by Minot and Cutler<sup>7</sup> are the only determinations on normal dogs that we have been able to find. Their average was 0.374, with a maximum of 0.48 and a minimum of 0.29.

Our experiments involved a study of 16 dogs. Of these, 8 showed either a definite decrease or no change in the guanidine content of the blood; 4 showed increase of less than 0.1 mg. in 24

<sup>1</sup> Burns and Sharpe, *Quar. J. Physiol.*, 1916, **10**, 345.

<sup>2</sup> Koch, *J. Biol. Chem.*, 1912, **12**, 313.

<sup>3</sup> Paton and Findley, *Quar. J. Physiol.*, 1916, **10**, 202, 315.

<sup>4</sup> Major and Weber, *Arch. Int. Med.*, 1927, **40**, 891.

<sup>5</sup> Ellsworth, *Johns Hopkins Hosp. Bull.*, 1930, **46**, 296.

<sup>6</sup> Minot and Cutler, *Proc. Soc. Exp. Biol. and Med.*, 1929, **26**, 607.

<sup>7</sup> Minot and Cutler, *J. Clin. Invest.*, 1928-29, **6**, 369.

<sup>8</sup> Simonds and Brandes, *Am. J. Physiol.*, 1925, **72**, 201.

<sup>9</sup> Major and Weber, *Johns Hopkins Hosp. Bull.*, 1927, **40**, 87; *Arch. Int. Med.*, 1927, **40**, 891.

hours with a return to normal in 48 hours; one showed a slight decrease in 24 hours with a return to approximately normal in 48 hours; while only 2 showed a definite increase (from 0.340 to 0.444 mg. and 0.340 to 0.466 mg., respectively). In each of these latter dogs peritonitis was present, and one of them being quite ill at the time it was sacrificed. One animal (No. 8) died 4 hours after the operation with symptoms of hypoglycemic convulsions; its blood sugar dropped from 104 mg. to 41 mg. and the guanidine from 0.409 to 0.337 during the 4 hours. All of these dogs showed liver injury on microscopic examination and most of them showed an increase in blood esterase.

From these experiments it appears that injury to the liver due to mechanical obstruction of the hepatic veins does not affect the guanidine content of the blood in the same manner as injury to the liver induced by acute poisons. It is suggested, although without any experimental basis, that the poisons used by Minot and Cutler may have injured the parathyroids as well as the liver.

## 5435

### Skin Reactivity of Mothers and Infants to *Staphylococcus Aureus* Filtrate and Vaccine.

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The negative response to the Dick and Schick tests in the new born and young infant gave rise to the supposition that neutralizing antibodies were present. These antibodies were considered to be of transplacental origin at birth, and then later might be obtained from the breast milk. Further clinical support is found in the apparent lack of diphtheria, scarlet fever, or other infectious diseases in early infancy. However, the Dick and Schick tests are often negative in the newborn although their mothers may be definitely positive (Cooke,<sup>1</sup> Ruh and McClelland<sup>2</sup>). Furthermore, the blood serum of the young infant also lacks the antitoxins that might neutralize the toxin of the scarlet fever streptococcus (Cooke<sup>1</sup>) or the diphtheria

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<sup>1</sup> Cooke, J. V., *Am. J. Dis. Child.*, 1927, **34**, 969.

<sup>2</sup> Ruh, H. C., and McClelland, J. E., *Am. J. Dis. Child.*, 1923, **25**, 59.

bacillus (Von Göer and Kassowitz,<sup>3</sup> Friedberger, Bock and Fürstenheim<sup>4</sup>). These antitoxins are usually found in the serum of adults who are negative to the Dick and Schick tests. In addition to the skin tests it has likewise been reported that the skin at this age responds neither to the filtrates from other specific organisms nor to certain non-specific antigens and eczematogenous irritants (Tschertkow,<sup>5</sup> Adelsberger,<sup>6</sup> Friedberger and Heim<sup>7</sup>).

There is still, however, a lack of study of skin response to antigens prepared from organisms to which the newborn and young infants are clinically very susceptible. This communication is limited to a report of the skin reactions after intradermal injections of *Staphylococcus aureus* filtrate and vaccine in mothers and their babies, as well as in older infants. This organism has been shown by Falls<sup>8</sup> and other investigators to cause pemphigus and impetigo in the newborn. The filtrate was prepared according to the method of Pilot and Afremow<sup>9</sup> and the organisms were obtained from boils, abscesses, puerperal sepsis, pemphigus, and impetigo. Thus a series of 46 mothers and babies were simultaneously injected with 0.1 cc. of 1:100 filtrate as soon after labor as possible. Thirty-four of these were also given injections of vaccines 0.1 cc. of 50 to 100,000,000 per cc. suspensions. The tested area was read and measured 20 to 24 hours later and only an erythema of 1 cm. in diameter or larger was considered as positive.

A large majority of the mothers responded with a definite reaction, some even as large as 3 to 4 cm. in diameter and in dilutions as high as 1:500. Their babies, however, gave little or no response. Another series of 82 infants, from one week to one year, were studied at the St. Vincent's Infant and Maternity Hospital. A tabulation of the entire series is appended to show the comparative reactivity of mothers and infants. In general, the latter respond very little in the first few months, and the proportion of positive reaction increases up to the end of the first year. The reactions were small in size and not much larger than 1 cm. in diameter. Those less than 1 cm. were considered negative, and there was a small number in this group with reactions of approximately 0.5

<sup>3</sup> Von Gröer, F., and Kassowitz, K. (quoted by Cooke).

<sup>4</sup> Friedberger, E., Bock, G., and Fürstenheim, A., *Z. Immun. forschg.*, 1929, **64**, 294.

<sup>5</sup> Tschertkow, L., *Z. Immun. forschg.*, 1929, **64**, 407.

<sup>6</sup> Adelsberger, L., *Z. f. Kinderh.*, 1927, **43**, 373.

<sup>7</sup> Friedberger, E., and Heim, F., *Deutsch. M. Wochenschr.*, 1929, **55**, 132.

<sup>8</sup> Falls, F. H., *J. Infect. Dis.*, 1917, **20**, 86.

<sup>9</sup> Pilot, I., and Afremow, M. L., *J. Am. Med. Assn.*, 1927, **89**, 939.

TABLE I.

Skin Reactions (24 hours) to Intradermal Injections of *Staphylococcus Aureus* Toxin and Vaccine.

	No. Tested	Reactions		% Positive
		Positive	Negative	
<i>Mothers</i>	T* 46	45	1	98.7
Several hours to 6 days puerperium	V† 34	30	4	88.5
<i>Newborn Babies</i>	T 46	0	46	0
Few hours to 5 days	V 34	1	33	2.9
<i>Infants</i>	T 39	1	38	2.5
1 week to 2 months	V 39	0	39	0
<i>Infants</i>	T 21	1	20	4.8
2 to 4 months	V 21	3	18	14.3
<i>Infants</i>	T 14	2	12	14.2
4 to 8 months	V 14	5	9	35.6
<i>Infants</i>	T 8	6	2	75.0
8 to 12 months	V 8	5	3	62.5

\* Toxin. † Vaccine.

cm. which were considered traumatic. These injections were entirely harmless to the mothers or babies, *i. e.*, no general reactions or skin complications followed.

Negative skin reactions were present in one case of pemphigus, 4 of generalized furunculosis, and 2 with abscesses. *Staphylococcus aureus* was isolated from the pemphigus and furunculosis babies. Further work on the skin reactivity of infants will be carried on with reference to gonococcus filtrate and other non-specific skin irritants. In the study of the sera of 15 mothers and babies who were positive and negative respectively to these tests, it was seen that the mother's opsonic index very frequently exceeded that of her baby (cord blood).

*Conclusions.* A report of skin reactivity to filtrate and vaccine of *Staphylococcus aureus*, which often causes infection in newborn, is presented. It includes a study of a series of mothers and their babies, and another of older infants. The response to intradermal injections of these preparations is parallel with the Dick, Schick, and other bacterial filtrates to which the skin of young infants is comparatively refractory. This lack of response is not associated with the presence of a protective mechanism, but rather, we believe, to an inherent lack of the reactive mechanism at this early age. Mothers and older infants give positive reactions in the proportions designated.

## Effect of Egg White in Oral Vaccination Against Pneumococcus.

HERBERT E. MC DANIELS. (Introduced by Lloyd Arnold.)

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Department of Bacteriology and Preventive Medicine, University of  
Illinois College of Medicine.*

The development of resistance to pneumococcus infection in young rats following the oral administration of various pneumococcus products has been reported by Ross.<sup>1</sup> Feeding of pneumococcus cultures to rats and rabbits was also done by Maeji<sup>2</sup> with similar results. That the mucous membrane of the gastro-intestinal tract is an effective site for the introduction of antigen, for the purpose of protecting the animal against pneumococcus infection, is also indicated in the work of Cooper<sup>3</sup> on sub-mucosal injection of vaccines in rabbits. Besredka<sup>4</sup> has shown that bile is useful in sensitizing or preparing the intestinal mucosa for the absorption of antigens; Arnold<sup>5</sup> and Finder<sup>6</sup> have investigated the effect of bile and egg-white on gastric secretion and on intestinal permeability to bacteria and antigens. The latter work shows that following the ingestion of egg-white and bile separately or in mixtures there is a definite lack of gastric response and an increased permeability of the small intestine.

The present work was undertaken (1) to confirm the experiments on oral immunization against pneumococci; (2) to simplify the procedure for producing an effective antigen in quantities suitable for human experiments; (3) to investigate the effect of preliminary preparation of the gastro-intestinal mucosa; and (4) to study the effect on pulmonary pneumococcus infections.

This report shows the effect of preparing the gastro-intestinal mucosa of the rat by feeding egg-white 30 minutes before the pneumococcus antigen is given. Young white rats of fairly uniform size (60-80 gm.) were fed 5 cc. of antigen; half of the animals received 5 cc. egg-white 30 minutes previous to the feeding of antigen. Five days later all animals were tested for immunity by intraperitoneal injection of 0.25 cc. of graded dilutions of broth culture of

<sup>1</sup> Ross, V., *J. Exp. Med.*, 1930, **51**, 585.

<sup>2</sup> Maeji, Y., *Acta Scholae med. univ. imp.*, Kioto, 1929, **12**, 295.

<sup>3</sup> Cooper, M., *J. Inf. Dis.*, 1926, **38**, 491.

<sup>4</sup> Besredka, A., "Local Immunization," Williams & Wilkins, 1927.

<sup>5</sup> Arnold, L., and Finder, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1928, **25**, 615.

<sup>6</sup> Finder, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 985.

the homologous type of virulent pneumococcus. At the same time, untreated animals were inoculated with the same organisms for the determination of the lethal dilution. In all experiments included in this resumé the lethal dose was found to be 0.25 cc. of a 1:100,000,000 dilution of an 18-hour culture of the type I strain used. The animals were fed and tested in 4 lots.

The toleration of many lethal doses (indicated by survival) was noted in all animals receiving antigen. If the maximum dose survived by animals in one group is taken as an index of the titer of the immunity developed in that group, we find results as indicated in the accompanying chart. The ordinates indicate the maximum

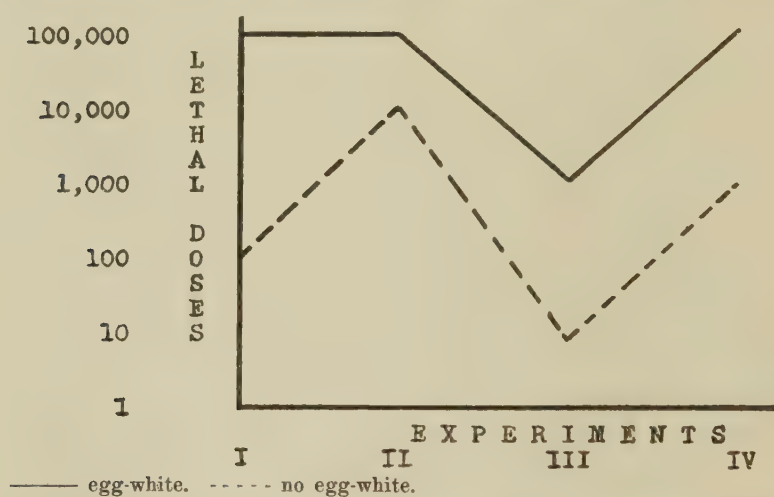


FIG. 1.

Effect of egg-white in oral vaccination against pneumococcus—4 experiments.

dose survived by each group; the abscissas indicate the separate experiments. It will be seen that all the animals developed a resistance to many lethal doses of the virulent culture. It is also clear that in every case those rats receiving a preliminary dose of egg-white exhibited a greater resistance to infection than those not fed egg-white.

Further comparative experiments of this sort are now being undertaken to find what effect egg-white has upon the duration of immunity and upon the rapidity of its appearance.

5437

**Destruction of Yeast in the Small and Large Intestines.**

B. E. MONTGOMERY, A. K. BOOR, L. ARNOLD AND O. BERGEIM.

*From the Laboratories of Bacteriology and Physiological Chemistry, University of Illinois College of Medicine.*

Different varieties of yeast were incubated with a mixture of human pancreatic juice and bile for 4 hours at 37.5°. From 50 to 99% of the yeast cells were killed. One variety of yeast showed a destruction of 50% in 16 hours and 81% at 48 hours, indicating that a part of the cells were more resistant than others or that the activity of the pancreatic juice decreased rapidly on standing. The pancreatic juice killed yeast least rapidly at about pH 7 and somewhat more rapidly in slightly acid solution (pH 6) than at pH 8.

Dogs were given 12 gm. of yeast with 100 cc. of water or 100 gm. of moist meat. The animals were killed at 2 or 4 hours after ingestion of yeast, different segments of the gastrointestinal tract tied off, and microscopical and plate counts of cells in each segment made. At 2 hours after taking yeast with water over 80% of the cells were found in the ileum. At 4 hours 70% were found in the cecum and colon and of these 40% were alive. When given with meat the recovery from cecum and colon at 4 hours was 40%, of which about 40% were living.

Destruction of yeast in different sections of the intestines of dogs was studied by injection of yeast into tied off segments in the living animal. No pronounced differences in rate of destruction in different parts of the intestine were noted nor any influence of reaction of injected material on such destruction. The per cent of yeast cells killed varied from 12 to 45%.

Yeast was fed in 12 gm. portions to normal men, 3 times a day with meals or with water before meals. The fecal excretion of live cells for 6 day periods varied from 0 to 19.5%, in most cases being below 1%. The excretion was quite variable from day to day and appeared to be influenced by the length of time the yeast remained in the large intestine. A slightly greater average excretion was noted when the yeast was given with water before meals.

Yeast incubated with fresh human feces was in most cases almost completely killed in 24 hours and a majority of cells were killed in 12 hours. This supports the view that there is a destructive agent in the contents of the large intestine which plays a prime rôle in determining the final degree of destruction of ingested yeast and

probably also of other micro-organisms. This substance is apparently produced by bacterial action and its nature is being studied.

## 5438

**Influence of High Protein Diets on the Kidney.**

R. KLEIN AND O. BERGEIM.

*From the Laboratory of Physiological Chemistry, University of Illinois College of Medicine.*

According to Newburgh and Curtis<sup>1</sup> the type of protein fed, with respect to its amino acid content, appeared to be the most important element in the nephropathogenic action of a high protein diet. Intestinal putrefaction, however, may also play a rôle. In attempting to study these factors 36 rats were divided into 9 groups for feeding purposes. The diets contained: Salt mixture, 4%; sucrose, 14 to 15%; agar, 2%; yeast powder, 8 to 10%; 140 mg. cod liver oil per rat per day; butter fat, 15%, except in diets VII, VIII and IX; starch to make 100 parts after protein was added. The diets contained protein as follows:

Group I. 20% casein (control).

Group II. 50% casein.

Group III. 30% egg albumin and 20% casein.

Group IV. 30% meat powder and 20% casein.

Group V. 30% powdered horn, 10% casein and 10% meat powder.

Group VI. 30% hydrolyzed horn, 10% casein and 10% meat powder.

Group VII. 60% casein and 10% meat powder.

Group VIII. 50% predigested casein, 10% casein and 10% meat powder.

Group IX. 60% casein (Harris) and 10% meat powder.

The horn was used because it was difficult to digest and should be the source of more putrefactive substances in the intestine. The hydrolyzed horn, predigested casein and finely divided casein (Harris) were used to see whether putting the proteins in a more rapidly absorbable form might have any effect.

One rat in each group was killed by chloroform after 19, 35, 70,

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<sup>1</sup> Newburgh and Curtis, *Arch. Int. Med.*, 1928, **42**, 801.

and 135 days of feeding and the kidneys removed, weighed, sectioned, stained and examined histologically.

Although the feeding time was short the results obtained were as follows:

1. The animals in Groups VII, VIII, and IX receiving 75% protein all showed some renal hypertrophy which, however, did not exceed 40% in any case.

2. Those rats receiving the meat powder, keratin, and 60% casein components in their diets for 35 to 137 days showed histological changes of the kidneys which were essentially tubular and consisted of degeneration of the tubular epithelium, swelling and degeneration of the nuclei and many tubular casts. The changes were most marked in the group receiving the meat powder.

3. Where the indigestible protein of horn was fed with presumably a considerable increase in intestinal putrefaction, differences were not shown that would indicate a marked influence of intestinal putrefaction on the kidneys under the conditions of the experiment.

4. The feeding of predigested casein (the hydrolyzed horn was discontinued because the animals refused to eat sufficiently of it) which should facilitate the absorption of the amino acids, did not appear to play a rôle in determining the effect on the kidney.

## Iowa Section.

*State University of Iowa, February 6, 1931.*

5439

### Colorimetric Studies on the Blood Exchange in Parabiotic Rats.\*

ROBERT T. HILL. (Introduced by E. Witschi.)

*From the Zoological Laboratory, State University of Iowa.*

While in cattle twins with anastomosing chorioallantoic blood vessels and in amphibians grafted together at an early embryonic stage sex hormones are exchanged in sufficiently large amounts to cause sex transformation, little, if any effect so far has been reported from experiments on parabiosis in fowl and in rats. This faces us with the double problem of the amounts of active hormones that may be transferred from one animal to its parabiotic twin and of the threshold values of the hormones considered. Witschi<sup>1</sup> states that the capillary connections in chains of a newt let pass enough of the hypophyseal hormones to stimulate the processes leading to metamorphosis, but too little to bring about the expansion of the melanophores.

From this it becomes obvious that the quantitative factor should be taken into consideration at the outset of parabiosis experiments. The author, therefore, has started some colorimetric studies on the amount and speed of exchange of blood in parabiotic rats. The technique developed by Dawson, Evans and Whipple<sup>2</sup> and by Smith<sup>3</sup> in their work on the blood volume of dogs has been adapted to the special conditions of our experiment.

One-tenth of a cubic centimeter of 1% Brilliant Vital Red (Evans) was injected into the heart of one of the twins. At the end of one hour samples of 3 to 4 cc. of blood were taken from both

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\* Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

<sup>1</sup> Witschi, E., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 763.

<sup>2</sup> Dawson, Evans and Whipple, *Am. J. Physiol.*, 1920, **51**, 232.

<sup>3</sup> Smith, H. P., *Bull. Johns Hopkins Hosp.*, 1925, **3**, 177.

members of the pair, mixed with oxalate solution and centrifuged. The clear dye-plasma-oxalate solution thus obtained was compared with a standard by use of the micro combination of the Leitz Universal Colorimeter. In all cases dealt with in this paper the parabiotic pairs consisted of 2 normal female litter-mates. The members of each pair were of practically the same size and so we may assume identity of blood volumes at the time of making the tests. Colorimetric readings, therefore, were simply calculated into percents to obtain the respective amount of dye circulating in each twin.

TABLE I.  
Percent of dye in each animal at the end of 1 hour.

Group No.	Uninjected animal	Injected animal	Age in days	No. of days in parabiosis
	%	%		
IX	12.1	87.9	225	191
X	12.9	87.1	225	190
XII	26.5	73.5	226	184
XIV	10.8	89.2	227	187
XV	11.9	88.1	243	184
XVI	35.5	64.5	226	160
XVII	12.8	87.2	218	159
XX	21.0	79.0	221	154
XXI	17.7	82.3	158	121
XXII	26.3	73.7	180	124
XXVI	22.2	77.8	180	121
XXVII	20.0	80.0	177	121
XXVIII	34.0	66.0	183	118
XXXI	21.3	78.7	172	105
XXXIII	10.9	89.1	139	105
XXXV	19.6	80.4	134	99
Average	19.7	80.3	196	145

Examination of Table I shows considerable variation in the amount of dye in the uninjected animal at the end of one hour, varying from 10.8% to 35.5%. There is apparently no relationship between the amount of dye in the uninjected animal and the length of time that the animals have been in parabiosis. It is quite surprising that on the average only about 20% of the dye is found in the uninjected animal at the end of one hour. This indicates that the rate of blood exchange is relatively low and that the vascular connections are of capillary nature only.

With respect to the pairs of normal females listed in Table I we may say that their oestrous cycles are not synchronized, though some interference is quite obvious.

## 5440

Pigmentary Reaction in *Rana Clamitans* Larvae Following Treatments With X-Rays.\*

WILLIAM T. LEVINE. (Introduced by E. Witschi.)

*From the Zoological Laboratory, State University of Iowa.*

Incidental to experiments on the effects of x-rays on the sex-glands of *Rana clamitans* tadpoles, our attention was drawn to certain conspicuous changes in the dorsal skin, especially the formation of whirls by the epidermal melanophores preceding their disintegration. The animals, after being narcotized in a 1:10,000 solution of water-soluble anesthesin, were placed on water-soaked cellulocotton and exposed to unfiltered x-rays. A Victor Snook unit, with a universal air-cooled broad focus Coolidge tube was used to produce x-rays of different qualities.

In the normal skin we find 2 types of brown melanophores: first, the asterisk-shaped pigment cells of the stratum corium (Figs. 2 and 6c),† which are distributed evenly over the back and flanks of the larvae. To them is due the general slate color of the dorsal skin (Fig. 1). Secondly, we find the filiform melanophores of the epidermis (Figs. 4 and 6e). They exhibit a remarkable tendency to form aggregations thus giving rise to the characteristic black spots on the back of the clamitans tadpole. It is well known that the so-called experimental albinism obtained upon hypophysectomy is largely due to the extreme contraction of the epidermal melanophores.

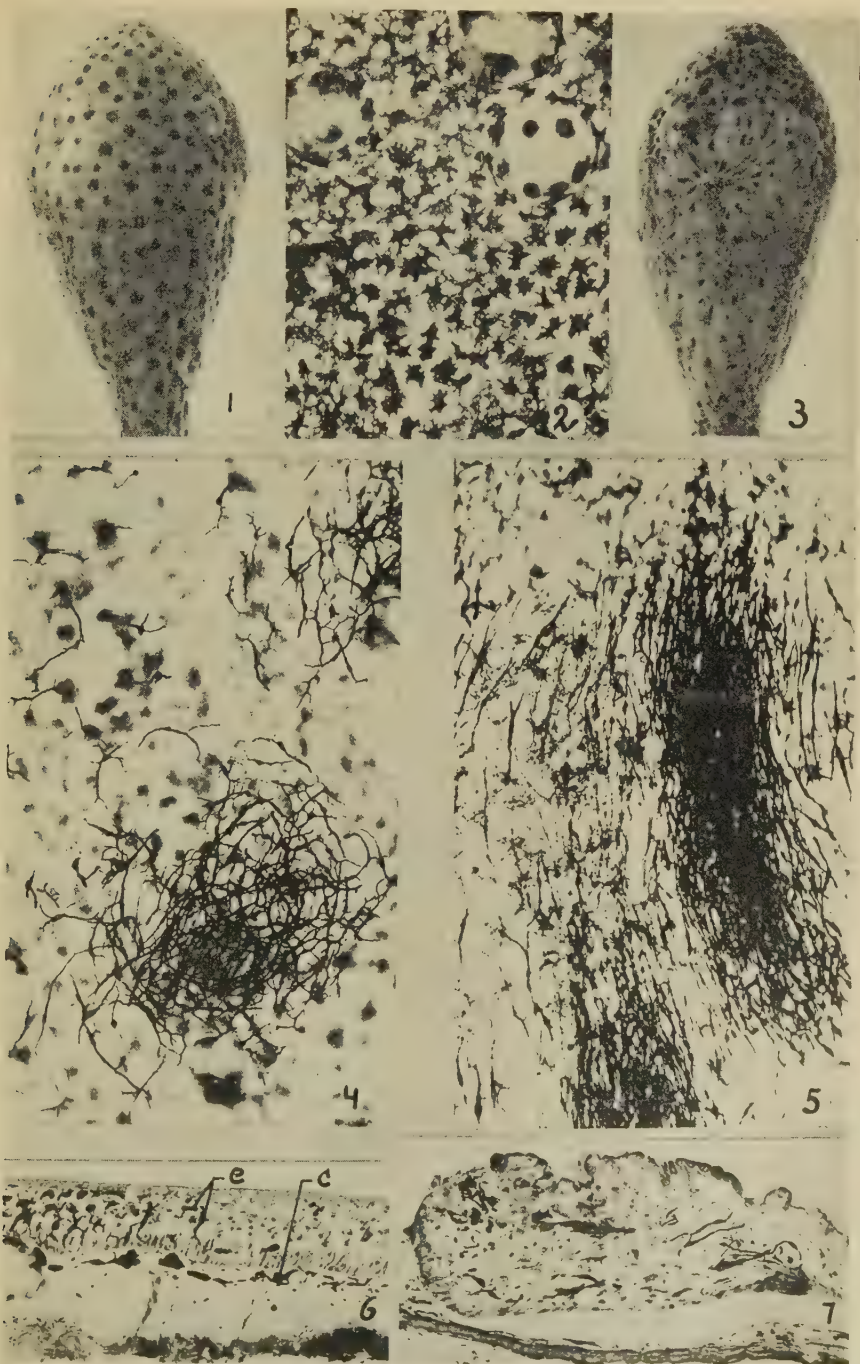
We describe here only 2 stages of the pigmentary reaction following x-ray treatment. In the first case 10 tadpoles, of 43-45 mm. body length, and 8-10 mm. hind leg length, were given 8 treatments of 92 kilovolts 5 milliamperes, at 10 inches from the cathode. The animals were exposed for 10 minutes every other day and killed 6 days after the last treatment.

Towards the end of the treatment the previously round pigment dots become streaky or comma-shaped. Later they begin to converge towards one or several centers which most often are located

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\* With the aid of the Committee on Effects of Radiation on Living Organisms of the National Research Council.

† Figs. 1 and 3 are only slightly enlarged photographs of living specimens. All the other figures are enlarged 85 times. Figs. 2, 4, and 5 are photomicrographs of whole mounts of skin, while 6 and 7 are taken from cross-sections. Figs. 1, 2, 4, and 6 show the condition of a normal animal, while 3, 5, and 7 illustrate the experimental reaction.



in the scapular and pelvic regions (Fig. 3, 22 days after the beginning of treatment). Here one observes a hyperplastic rise of the epidermis and eventually a perforation appears in the center, plugged with a blood clot. Examination of sections (Fig. 7), and whole mounts (Fig. 5) shows that the epidermal melanophores, normally irregularly arranged (Fig. 4), have become oriented in one direction. Evidently, they are making use of their power of ameboid movement and seem to proceed towards the most impaired places. However, on approaching the centers of injury they succumb soon themselves. They contract and subsequently break up into a number of black droplets. The successive stages of this process may be seen in the section reproduced in Fig. 7. The extreme left corresponds to the center of the whirl of Fig. 3. The deeper melanophores of the stratum corium exhibit no marked changes.

A second group of 10 tadpoles was exposed on 2 consecutive days for 45 minutes at 125 kilovolts 5 milliamperes at a distance of 10 inches. One or 2 days after the second treatment they present not only a formation of whirls, but also a noticeable decrease in epidermal pigments. After the second day the epidermis becomes highly abnormal and shows a tendency towards a sloughing off, leaving the stratum corium exposed. This gives the animal a slate colored appearance.

The described reaction of the epidermal melanophores serves as a sensitive indicator of the destructive processes caused in the skin by the exposure to x-rays. The histological changes in the epidermis of clamitans larvae correspond closely to those described by Colwell and Thomson<sup>1</sup> for an unidentified species (probably *Rana temporaria*). In our material, however, the presence of epidermal melanophores renders the progress of the pathological reactions macroscopically visible. Active wandering of melanophores in the epidermis of Californian tadpoles has been observed by Smith<sup>2</sup> in skin transplants. Any mechanical injury to the dorsal skin of our species initiates similar movements of the nearby pigment cells. It is evident, therefore, that the pigmentary dislocations are not specific to external stimuli but rather to internal disturbances due to various causes.

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<sup>1</sup> Colwell, Hector A., and Thomson, M. Sydney, *Am. J. Roentgen. and Rad. Ther.*, 1927, **17**, 1.

<sup>2</sup> Smith, Philip E., *Am. Anat. Mem.*, 1920, **11**.

5441

Rôle of Proteins in Growth, Reproduction and Lactation.  
I. Beef Liver.

H. GREGG SMITH. (Introduced by H. A. Mattill.)

*From the Biochemical Laboratories, State University of Iowa.*

In previous studies of growth and reproduction of white rats on meat diets, whole raw, cooked or cooked and dried tissues have been used. Since the biological differences between such diets might reside not only in the protein but also in the tissue lipids, a series of experiments has been planned in which tissues extracted with alcohol until fat-free form the source of protein.

In these preliminary experiments on liver, the protein was fed at 2 levels, 15% (diet A) and 20% (diet B). In addition the diets contained 15% fat (hydrogenated cottonseed oil), 4% salts, 2% agar and starch to make 100%. Yeast and cod-liver oil were administered daily.

First generation animals on diet B showed a normal growth rate, while growth on diet A was considerably below normal. Three females on diet A gave birth to 11 litters containing a total of 73 young and 12 litters containing 84 young were born to 3 females on diet B. Lactation was deficient and only 40% of the young were weaned on each diet. The mothers lost considerable weight during the nursing period and the young animals averaged only 26 and 28 gm. in weight, respectively on the two diets, at 21 days of age.

Growth of the second generation animals was very much inferior to that of the first generation, averaging 25% less on both diets. Animals which received daily one-half gram of whole dried liver grew at a much faster rate than control animals. The increased growth was not related to the vitamin content of the liver. The reproductive function of the females was abnormal. Thirteen females on the 2 diets gave birth to 18 litters containing 109 young and showed an almost total inability to rear them, all but one having died within a few days after birth. Lactation was not improved by increased amounts of vitamins A and B. The majority of the second generation males were sterile and the oestrus cycles of the females were irregular.

Explanations for this beginning sterility and for the growth-accelerating effect of the whole liver are being sought.

Response of the Heart to Exercise of Graded Intensity.

W. W. TUTTLE.

*From the Department of Physiology, State University of Iowa.*

Changes in the heart rate produced by different degrees of activity have been used as a basis in many of the tests of physical efficiency. If the changes in heart rate are not directly proportional to the amount of exercise performed, then tests of physical efficiency based on this principle are not reliable.

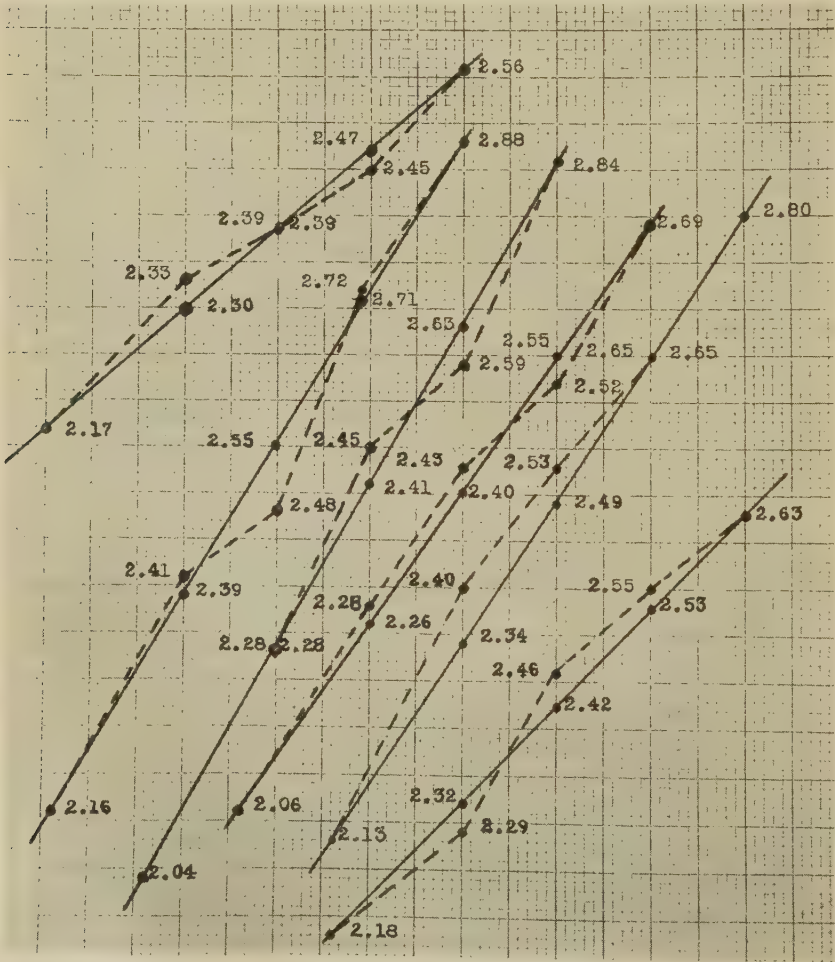


FIG. 1.

Data obtained from 6 typical cases of a group of 75 which have been examined are presented to show the frequency response of the heart to exercise of graded intensities.

The frequency responses of the heart are based on the pulse-ratio. This is determined by dividing the pulse for 2 minutes after a known amount of exercise by the normal sitting pulse for one minute.

The exercise consists of mounting a stool 13 inches high a definite number of times in one minute. Suppose, for example, that after mounting the stool 18 times in one minute the resulting pulse-rate for 2 minutes is 135. Assuming the normal pulse to be 60, then 18 steps of the exercise produce a pulse-ratio of 2.25.

If the response of the heart as indicated by the pulse-ratio is directly proportional to the amount of exercise performed, then the curve which results from plotting a number of pulse-ratios of an individual against the amount of work done to produce them, is a straight line. The results of such an experiment involving 6 subjects are shown in Fig. 1. In each case, 18 steps is the initial exercise and 40 steps the most strenuous.

If, as is the case with one of the subjects, 18 steps per minute produce a pulse-ratio of 2.17 and 40 steps give 2.63, then by the graphic method, 25 steps should produce a pulse-ratio of 2.33, 30 steps 2.43 and 35 steps 2.53. By actual performance 25, 30 and 35 steps produced pulse ratios of 2.29, 2.46 and 2.55. The differences between the plotted and experimental values are 0.04, 0.03, and 0.02. A comparison of the plotted and experimental values is shown in the figure.

The differences between the plotted and experimental values as shown in the figure appear large but this is due to the fact that the pulse-ratios are plotted to the scale 0.01 pulse-ratio equals 1 mm. on the ordinate and 2 mm. on the abscissa equals one step. The greatest difference between plotted and experimental values is 0.07 of a pulse-ratio which represents a difference of 2 steps per minute. A difference as large as this is well within experimental error. An error in counting amounting to 4 beats in 2 minutes is sufficient to account for all of this difference. Furthermore, an error of from one to 2 beats per minute in counting the normal pulse is sufficient to produce the difference.

The results of an experiment which covers a study of 75 cases of which only 6 are reported, show that the increase in the rate of the heart beat is directly proportional to the amount of exercise performed.

## Pacific Coast Section.

*Stanford University School of Medicine, February 18, 1931.*

5443

### Transplants of Adrenal Cortex Into Rat Ovaries.

R. I. PENCHARZ AND J. M. D. OLMSTED.

*From the Department of Zoology and the Division of Physiology, University of California.*

We have reported<sup>1</sup> that transplantation of adrenal cortex into the ovary at the same time that bilateral adrenalectomy was performed proved successful in maintaining the life in 5 out of 12 piebald rats, and suggested<sup>2</sup> that if the operation were carried out in 2 stages some weeks apart—(a) removal of one adrenal and transplantation of a portion of it into an ovary, (b) removal of the second adrenal

TABLE I.

Rat	Date of 1st stage of oper.	Weight at 1st stage gm.	Date of 2nd stage of oper.	Date of removal of transplant	Weight at time of removal of transplant gm.	Date of death following insufficiency symptoms
W 5462*	9/29/30	148	10/20/30	12/15/30	210	12/18/30
G 5900	10/28/30	127	11/12/30	1/ 2/31	200	1/12/31
B 5901	10/28/30	143	11/12/30	1/ 2/31	205	1/15/31
BH 5902	10/28/30	114	11/12/30	1/ 2/31	180	1/13/31
BH 6202†	11/ 4/30	128	11/20/30	1/21/31	251	2/ 4/31
G 5801‡	10/27/30	203	11/13/30	1/21/31	204	—

<sup>1</sup> Pencharz, R. I., Olmsted, J. M. D., and Giragossintz, G., *Science*, 1930, **72**, 175.

<sup>2</sup> Pencharz, R. I., Olmsted, J. M. D., and Giragossintz, G., in press.

\* Mated 11/27/30. Note that following removal of transplant pregnancy did not prevent insufficiency symptoms and death.

† Mated 11/12/30. Gave birth to 11 young 12/13/30.

‡ Mated 11/19/30. Litter of 8, 12/12/30. Inasmuch as this rat failed to show insufficiency symptoms following removal of transplant, it was killed 2/8/31. An accessory adrenal was found. This is only the second case of an undoubted accessory found by us.

some time following the first operation—we believed the operation would be successful in a greater number of cases. In this paper we report that in 6 attempts at transplantations with this method all were successful.

All the rats used in the above experiments were virgin animals, 3 to 5 months old at the time of the initial operations, and all transplants were made during dioestrous. The transplant was successful in each case. All 6 rats were in excellent health and showed no insufficiency symptoms at the time of removal of the ovary containing the adrenal transplant 50 to a little over 60 days following removal of the second adrenal, *i. e.*, a much longer interval than the longest survival period of our successfully adrenalectomized rats;<sup>2</sup> all except one, in which accessory adrenal tissue was found, showed typical insufficiency symptoms and died 3 to 13 days after removal of the transplant. It is to be noted that the operation did not interfere with normal pregnancy, and that pregnancy did not prevent insufficiency symptoms and death when the transplant was removed.

#### 5444

### A New Method of Determining Solubilities Based on Stability of Phthalate Buffers of Low pH at Low Temperatures.

J. P. BAUMBERGER AND F. A. DAVIDSON.

*From the Physiological Laboratory of Stanford University.*

The disconcerting finding that buffers of potassium acid phthalate-HCl become more alkaline on standing led to a consideration of the cause of this change and to the discovery of a new method of determining solubilities.

The buffers were made according to the directions given by Clark.<sup>1</sup> The immediate determination of the pH of these buffers by the quinhydrone electrode gave values close to those expected, but subsequent pH determinations showed that a change had taken place on standing. This was particularly true at 13°C., whereas at 20°C. the changes were not great. A deposition of crystals could be observed accompanying the change in the pH of the buffers and this crystallization seemed to be proportional to the degree of change in the pH values. After the effect had been noticed in one set of buffers a new series was made up and the pH values determined by

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<sup>1</sup> Clark, W. M., Determination of Hydrogen Ions. 1928.

the quinhydrone electrode within 2 hours after making it up. Each buffer in the series was then divided into 2 pyrex flasks. One set was placed at 20°C. and the other at 13°C. for 48 hours after which the pH values were again determined. The results are shown in Table I.

TABLE I.  
Experiments at 20°C.

Determined pH		$C_{H+} \times 10^{-2}$	Molar Concentration. Undissociated Phthalic Acid.		
2 Hours A	48 Hours B		2 Hours C	48 Hours D	Change C—D
2.26	2.33*	— .081	.041	.040	— .001
2.44	2.50*	— .047	.038	.036	— .002
2.64	2.66	— .010	.033	.032	— .001
2.74	2.70	+ .018	.030	.031	+ .001
2.80	2.79	+ .004	.028	.029	+ .001
3.97	3.97	.000			

Experiments at 13°C.

2.26	2.49*	— .225	.041	.037	— .004
2.44	2.64*	— .134	.038	.033	— .005
2.64	2.73*	— .043	.033	.031	— .002
2.74	2.79	— .020	.030	.029	— .001
2.80	2.89	— .029	.029	.026	— .003
3.97	3.97	.000			

\* Crystals.

The results show that the pH changed appreciably at 20°C. in buffers of pH 2.26 and 2.44 but not in the buffers of higher pH value. At 13°C. the pH changed considerably more in each of the above mentioned buffers as well as changing definitely in the buffers up to pH 2.80. The exact change in  $C_{H+}$  after 48 hours is shown in column 3 of the table. The buffers showing crystal formation are indicated in the table by the asterisk (\*) and seem to coincide with high values for change in  $C_{H+}$ .

The changes in the pH values noted above are to be expected from the solubility of phthalic acid as given by Seidell.<sup>2</sup> The solubility of phthalic acid as determined by the content of a saturated solution is approximately 6.2 gm. per liter at 20°C. and 5.2 gm. per liter at 13°C. The solubility of the salt of phthalic acid, however, is very high. The  $C_{H+}$  of the pure acid may be given approximately by the following equation:

<sup>2</sup> Seidell, A., *Solubility of Inorganic and Organic Compounds*, 1919, I, 490; 1928, II, 1347.

$$(1) \quad C_H + = \sqrt{K_1 \cdot \text{Conc. of Acid.}}$$

At 13°C. the total concentration of the undissociated acid will be  $1-\alpha$  and assuming  $pK_1 = 2.94$  at 13°C. and 2.93 at 20°C. we have

$$(2) \quad pH \text{ } 13^\circ C. - pK_1 13^\circ C. = \log \frac{\alpha}{1-\alpha}; \quad 2.22 - 2.94 = \log \frac{\alpha}{1-\alpha}, \quad 1-\alpha = 84\%.$$

$$\text{Similarly at } 20^\circ C.; \dots\dots\dots 2.18 - 2.93 = \log \frac{\alpha}{1-\alpha}, \quad 1-\alpha = 85\%.$$

The undissociated acid of a saturated solution of phthalic acid would be 0.026 Molar at 13°C. and 0.031 Molar at 20°C. In the M/20 acid phthalate buffers used above, the concentration of undissociated phthalic acid may be calculated from the pH and total concentration of phthalate by using equation (2). The values obtained are shown in the fourth and fifth columns of the table. In the experiments at 20°C. there is a definite tendency for the phthalic acid to crystallize out until a final saturation value of 0.031 Molar is reached, whereas at 13°C. the process is more extensive and approaches the saturation value of 0.026 Molar. That a final end to this process had not come in the 48 hours is evident from the fact that buffers of pH 2.2 and 2.5 changed to pH 2.46 and 2.76 respectively on standing several weeks at 13°C.

This paper points out (1) that M/20 acid phthalate buffers of pH values less than 2.7 are supersaturated with phthalic acid at 20°C. and buffers of pH values less than 2.9 are supersaturated at 13°C. Consequently these buffers do not have a stable pH value and may be a source of considerable error where work is carried on at temperatures below 25°C. As this buffer system is extensively employed in biological work the above dangers are noteworthy.

(2) The solubility of the weak acid or the weak base employed in buffers as affected by changes in temperature and concentration may be accurately determined by a study of the accompanying change in the pH values; thus, a new method of solubility determination is here indicated.

## Effect of Hyperpyrexia Produced by Baths upon the Intracranial Pressure in Epileptics.

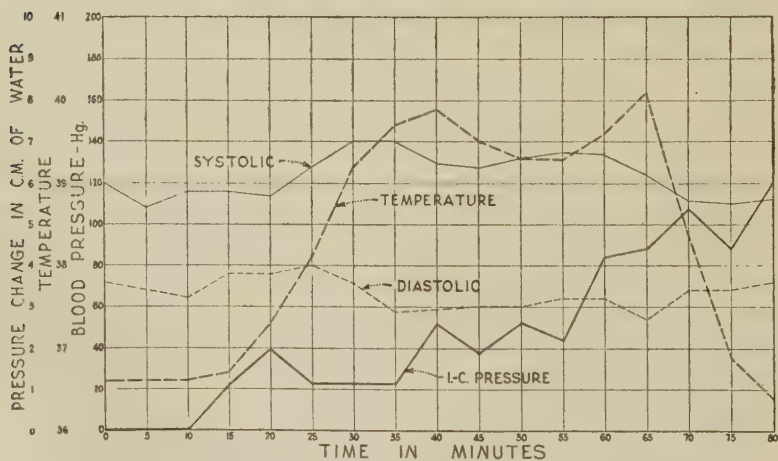
HENRY G. MEHRTENS AND W. LYLE ALLRED.

*From the Neuropsychiatric Division, Stanford University Medical School.*

Hyperpyrexia produced by baths is now frequently utilized in many neurologic disorders.<sup>1</sup> Epileptics, when mouth temperatures are raised to 39.5°C. or higher, are especially liable to convulsive attacks while in the bath. The question of changes in intracranial pressure was investigated.

In attempting to get direct information on the subject, the method of Stevenson<sup>2</sup> *et al* for measuring changes of intracranial pressure seemed the most available. Their method consists of utilizing the cerebral hernia following subtemporal decompression. Their apparatus consists of a closed air tambour system under a positive pressure of 12 mm. of mercury. One tambour was strapped over the brain hernia. The other was attached to a pointer which registered on a smoked drum the changes in intracranial pressure.

For our purpose, when the patient was subjected to high temper-



D-INTRACRANIAL PRESSURE CHANGES  
DURING HYPERPYREXIA BATH

CHART A.

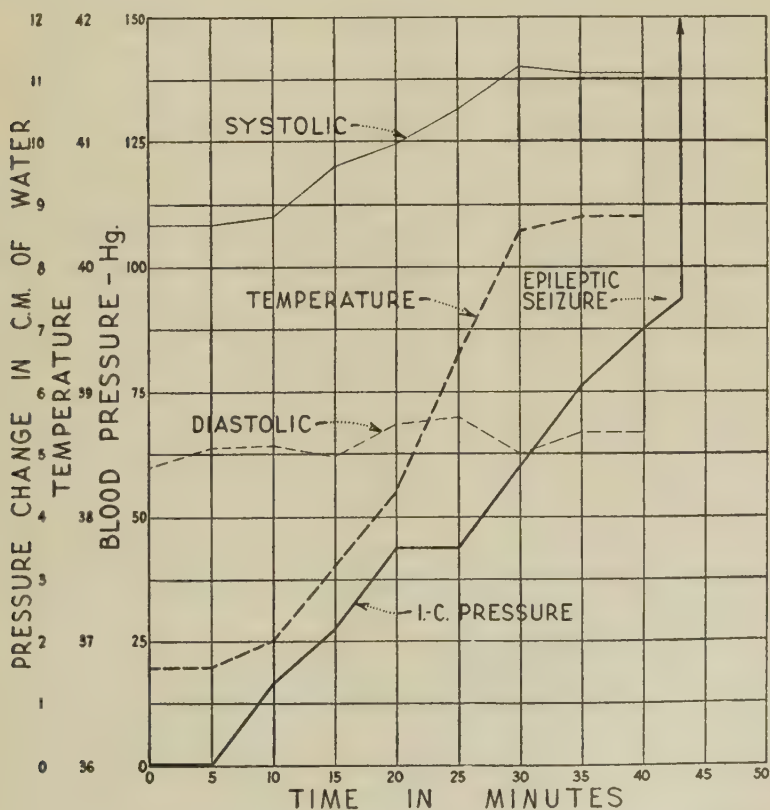
Effect of hyperpyrexia produced by baths upon intracranial pressure in epileptics.

<sup>1</sup> Mehrtens and Pouppirt, *Arch. Neurol. and Psy.*, 1929, **22**, 700.

<sup>2</sup> Stevenson, Lewis, Christensen, B. E., and Wortis, S. Bernard, *Am. J. Med. Sciences*, 1929, **178**, 663.

ature with consequent involuntary movements, it seemed desirable to modify the above method. The tambour, strapped over the hernia, was attached to a closed manometer system and the pressure changes read directly on the U tube of the manometer. The patient was placed in the continuous bath at  $37^{\circ}\text{C}.$ , a control observation made for 20 minutes, then the hot water in the tub was raised to  $41^{\circ}\text{C}.$  until the mouth temperature of the patient was raised to  $40^{\circ}\text{C}.$  This mouth temperature was maintained about 20 minutes.

Five such observations were made on 2 epileptic patients. Chart A shows a gradual rise in intracranial pressure as the mouth temperature increased, but not accompanied by a corresponding increase in blood pressure.



### D-INTRACRANIAL PRESSURE CHANGES DURING HYPERPYREXIA BATH

CHART B.

Effect of hyperpyrexia produced by baths upon intracranial pressure in epileptics.

Chart B shows a condition—occurring twice in one series—in which the intracranial pressure increased rapidly upon applying hyperpyrexia, and ended in an epileptic convulsion.

*Conclusions.* Hyperpyrexia produced by baths raises the intracranial pressure in epileptics, and perhaps in normal individuals. The hyperpyrexia frequently results in an epileptic seizure, occurring as the intracranial pressure rapidly rises. The seizure may be related to this rapidly increased intracranial pressure. Normal individuals never develop convulsions when subjected to such hyperpyrexia.

## 5446

### Survival of Bacteriophage.

M. S. MARSHALL AND F. S. PAINE.

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The interval of time over which bacteriophage filtrates maintain their activity is of general biologic interest. Enzymes demonstrate their activities after many months. Immune substances retain their activities for at least more than 4 years. Ultramicroscopic viruses retain their activity under certain circumstances for several years. Ballantyne<sup>1</sup> has shown the survival of bacteria in water for at least 32 months. Anaerobic organisms have been recovered from old cultures after more than 14 years. The plague bacillus has remained viable for 10 years. Cultures of *Leptospira icteroides* and *L. icterohemorrhagiae* may show motility after 2 years and 4 months (Hadley, P.). Pure cultures of molds have yielded active spores after longer periods. The bacteriophage has been demonstrated to retain its activity for more than 5 years.

The present brief note is based on tests of a bacteriophage filtrate originally recovered by d'Herelle. It was sent to Dr. F. G. Novy in 1921, and was rejuvenated by Dr. Philip Hadley against the Shiga dysentery bacillus for which it was said to be specific. The properties were specifically studied<sup>2</sup> during the fall and winter of 1922-23, during which time several filtrates were sealed in tubes and stored in the dark at room temperature. The menstruum was a beef infusion broth, presumably pH  $7.2 \pm 0.2$ , used for all of the work

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<sup>1</sup> Ballantyne, E. N., *J. Bact.*, 1930, **19**, 303.

<sup>2</sup> Marshall, M. S., *J. Infect. Dis.*, 1925, **37**, 126.

performed. Tubes were sealed in a flame (glass seal), allowing about 4 cc. of air space for 10 cc. of filtrate. They have thus been held until the present time, now 8 years. The filtrate used was water clear, although very light granular precipitate appears in several of the ampoules not opened.

Recent tests indicate a weakened, but readily revived activity, which is apparently identical with the original filtrate. One loop of the original filtrate failed to inhibit 14 dysentery strains. A second test, using 2 drops, checked the growth of one Flexner strain. A filtrate of this tube partially inhibited 13 of the 14 strains at hand, using 1 drop. The failure was a Shiga type. A pooled filtrate from the above 13 tubes was decidedly active against a representative Flexner, Hiss-Russell Y, and a Shiga type. A series of dilutions for titration by inhibition showed complete lysis in a dilution of 1:100,000, and partial but marked lysis in a dilution of 1:10,000,000. Higher dilutions presented normal growth. Plates made by a technic identical with that used 8 years ago not only showed a count of lysed areas still consistent with the inhibition-titration method with this bacteriophage, but the areas were similar in type. All areas ranged in diameter between 0.2 and 0.1 mm. and were visible with a hand lens. The lysed areas of the original material at the time were only slightly larger, and comparison with a photograph indicated that it was virtually impossible to distinguish between this and present plates. Although the smallest areas were not easy to locate, they were distinct, and microscopic examination failed to reveal areas not visible with an ordinary 3.5x hand lens. With a less complete check of the maintenance of specific properties, direct lysis from sealed tubes of this bacteriophage was last previously demonstrated after nearly 7 years.

The bacteriophage may, then, survive for at least 8 years, without materially changing its chief characteristics. There is no direct estimate of degree of degeneration, but it would seem probable that the curve of degeneration is at present reasonably flat, and that survival for some years further may be expected. The meaning of such survival, relative to the nature and characteristics of the bacteriophage, enters a speculative field which is enticing, but which has no place here.

## Experimental Therapy in Coccidiosis of the Domestic Fowl.

CARROLL NEFF. (Introduced by C. D. Leake.)

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In attempting to evaluate the relative therapeutic merits of various types of chemicals proposed from time to time as effective in coccidiosis of the domestic fowl (*Gallus gallus*), several such agents have been administered by mouth to naturally infested fowls, and the effects have been compared with the results obtained by rendering the intestinal contents more acid than normal by dietary regulation or by lactic acid. The hens studied were naturally infested with *Eimeria acervulina* Tyzzer, 1929, *E. maxima* Tyzzer, 1929, and *E. mitis* Tyzzer, 1929.

Diphenylamine and 3-acetyl-amino-4-hydroxyphenylarsonic acid (acetarsone N.N.R.) in 8 hens each were ineffective in varying doses by mouth in causing a cessation or decrease in the intensity of the discharge of oocysts of the organisms noted. These compounds failed to influence in any way the symptoms of the disease in the hens treated. Carbon tetrachloride administered by mouth in varying doses to 17 naturally infested hens was likewise without effect.

Tetrachlorethylene in doses of 1 cc. daily for 5, 7, and 12 days respectively in 3 infested hens had no effect. In daily doses of 2 cc. the drug caused a pronounced decrease in the intensity of discharge of all the oocysts involved within 5 days after treatment was inaugurated in the 4 hens studied. In doses of 2 cc. twice daily for 4 days the drug was also effective in reducing the intensity of the discharge of the oocysts. Tetrachlorethylene, however, when given to hens over a period of time causes characteristic lesions of the intestinal tract with pronounced symptoms and may even cause death.

Methyl violet at a dosage of 0.2 gm. twice daily for 2 days, while stopping the discharge of oocysts in the 3 hens studied, caused no improvement in the condition of the birds and, in fact, resulted in death 4 days after treatment was instituted. At postmortem examination very few oocysts were found in the intestinal tract of these hens.

An emulsion of lactic acid in agar, mineral oil, and water (furnished by the Kelp-ol Laboratories, Los Angeles) has been found to render the feces of certain animals more acid.<sup>1</sup> Such an emulsion

<sup>1</sup> Kessel, J. F., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 113.

lowers the pH of the feces of hens from a range of 7.1 to 6.4 to a range of 6.1 to 5.3, depending on the dosage and the period of time over which it is given. In 9 infested hens to which the emulsion was given for a period of time sufficient to cause a similar lowering of the pH of the feces, a complete cessation of the discharge of oocysts was noted in 5 cases and a reduction in the intensity of the oocysts discharge was observed in the other 4.

*Summary.* Lowering of fecal pH, in hens naturally infested with *Eimeria*, by means of an emulsion of lactic acid in agar, mineral oil, and water has been found to be more satisfactory in reducing or stopping oocyst discharge than the administration of various drugs, including carbon tetrachloride, tetrachlorethylene, methyl violet, acetarsone, and diphenylamine.

## 5448

### Oral Toxicity of Certain Alkyl Resorcinols in Guinea Pigs and Rabbits.\*

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Two alkyl resorcinols have recently been discussed in relation to the treatment of certain parasitic infestations: Lamson *et al*<sup>1</sup> suggesting hexylresorcinol in ascariasis and uncinariasis, and Faust<sup>2</sup> and Ratcliffe<sup>3</sup> proposing heptylresorcinol ("di-hydranol") particularly in amebiasis. We believe that clinical trial of new drugs in man may proceed with greater satisfaction than otherwise, if it is made after a critical study of the toxicity of such agents in various genera of mammals for the purpose of reaching an approximate quantitative estimate of the *toxic range* of the materials, and of determining what pathological effects for which it may be expedient

\* Part of a cooperative study of the chemotherapy of amebiasis conducted by the Pacific Institute of Tropical Medicine of the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by Eli Lilly and Co., Indianapolis, and the Ciba Co., Inc., New York City.

<sup>1</sup> Lamson, P. D., Wood, C. B., and Brown, H. W., *PROC. SOC. EXP. BIOL. AND MED.*, 1930, **27**, 1017; Lamson, P. D., Brown, H. W., Ward, C. B., Robbins, B. H., *Ibid.*, 1930, **28**, 191.

<sup>2</sup> Faust, E. C., *PROC. SOC. EXP. BIOL. AND MED.*, 1930 **27**, 905.

<sup>3</sup> Ratcliffe, H. L., *J. Parasit.*, 1930, **17**, 113.

to watch when used clinically and against which to guard. The data presented by Leonard and his associates,<sup>4</sup> while indicating that the toxicity of these drugs is "clinically negligible," do not seem to have been derived from a critical quantitative study. There is, however, a more significant reason from theoretical grounds for investigating quantitatively the toxicity of the alkyl resorcinols; in many series of related compounds containing alkyl radicals, it has been observed that toxicity in the series increases with increase in the number of carbon atoms in the straight carbon chain, and it is desirable to ascertain to what extent these observations may be expanded into a "law" relating chemical constitution to pharmacological action.

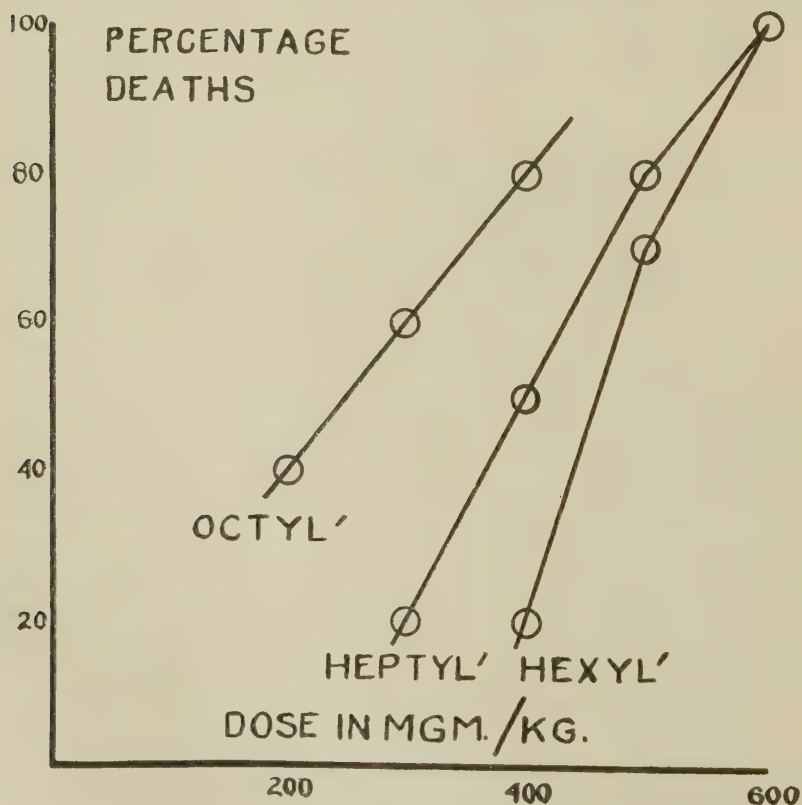


FIG. 1.

Graphical representation of the toxic range of hexyl-, heptyl-, and octyl-resorcinol in single oral doses to guinea pigs. Ten or more animals were used at each point indicated by a circle on the graphs.

<sup>4</sup> Leonard, V., *J. Am. Med. Assn.*, 1924, **83**, 2005; *J. Urol.*, 1924, **12**, 585; Leonard, V., and Feirer, W. A., in press.

The toxic ranges of crystalline hexyl-, heptyl-, and octyl-resorcinol (kindly furnished by Dr. Veader Leonard) in single oral doses in 115 guinea pigs were estimated by a general technique discussed elsewhere.<sup>5</sup> The animals were observed for 15 days after administration, and were compared with controls kept under similar conditions. The lethal range for guinea pigs of the 3 compounds is indicated graphically in Fig. 1. A definite increase in toxicity and a widening of the toxic range occurs with increase in the number of carbon atoms in the alkyl radical. The average lethal dose or M.L.D. of each respective drug may be estimated by dropping a perpendicular to the base line from the 50% death point on the graph of its lethal range.

Our toxicity studies in rabbits did not include enough animals to permit of a similar comparison to be made of these agents. We found in 6 rabbits that a single oral dose of 750 mgm. per kg. of hexylresorcinol in 10% concentration in ethylene glycol was tolerated with no significant effect except a transitory loss of weight and slight diarrhea. But 6 other rabbits, to which equivalent oral doses were given of the drug in crystalline form in gelatine capsules, all died within 5 days. This confirms Lamson's belief that the drug in solution is less toxic than when crystals are given. Three rabbits survived the oral administration of 750 mgm. per kg. of heptylresorcinol in 5% solution in olive oil with only slight symptoms of anorexia and loss of weight, but 3 others died within 5 days after giving 1 gm. per kg. of the same solution of the drug. In 5 equally divided doses at 2 day intervals, 3 rabbits tolerated 1500 mgm. per kg. of the same material with symptoms of diarrhea and loss of weight. Octylresorcinol in crystalline form killed 2 of 3 rabbits at 500 mgm. per kg. by mouth. While there is some evidence, therefore, in rabbits of increasing toxicity with increase in carbon content of the alkyl side chain in this series of related compounds, it is not as definite as in guinea pigs.

In lethal doses of these resorcinol derivatives, rabbits lost about one-third their original body-weight, and anorexia, lethargy, and diarrhea were noted. Immediately before death the animals became comatose, appeared very weak and emaciated, muscles were stiff, eyes were sunken, ears were drooped, and reflexes were sluggish. Respirations in one animal fell to 54 per minute, temperature was 34.2°C. and pulse rate was 114 per minute. At necropsy the animals usually were found with the liver moderately congested, the

<sup>5</sup> Burn, J. H., *Physiol. Rev.*, 1930, **10**, 146; Anderson, H. H., and Leake, C. D., *Am. J. Trop. Med.*, 1930, **10**, 249.

gall bladder distended, the stomach full with erosion of the mucous membranes, and with a few small petechial hemorrhages in the intestines. The kidney was the only tissue showing definite microscopic abnormalities. Dr. Stacy Mettier noted numerous areas of focal necrosis, with extensive hyaline degeneration of the epithelial cells lining the tubules, and with some desquamation of these cells. All 3 compounds in crystalline form are irritating to the mucous membranes of the eyes of rabbits, causing a marked pan-ophthalmitis within 24 to 48 hours after application of a single small flake. Octylresorcinol is very irritating to the skin and mucous membranes of the face and hands of one of us, producing a vesicular dermatitis on parts of the skin coming in contact with the agent.

## 5449

**Comparative Actions of Sympathomimetic Compounds: Responses of Cocainized Rabbit's Intestine.**

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*From the Department of Pharmacology, Stanford University School of Medicine.*

The extensive use by Tainter and others of the cocaine sensitization-desensitization phenomena in studies of the circulatory actions of sympathomimetic compounds, made it desirable to determine whether the same phenomena could be elicited with intestinal muscle. Some data were available in papers by Tainter and Chang<sup>1</sup> and Thienes and Hockett.<sup>2</sup> In no case, however, had the study of excised intestine been sufficiently extensive to indicate the range of spontaneous variation in the responses to successive administrations of the amines. We, therefore, investigated the influence of cocaine upon the responses of excised rabbit duodenum to epinephrine, tyramine, ephedrine and barium, controlling the results by observations of the responses to repeated applications of the same drug in the absence of cocaine. The changes in tonus, amplitude, and rhythmic activity were correlated as much as possible.

The tissues were fresh and active, the majority being taken from recently killed rabbits. Some tissues, however, were kept on ice for 24 hours; this procedure has been found by Burn and Tain-

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<sup>1</sup> Tainter, M. L., and Chang, D. K., *J. Pharm. Exp. Therap.*, 1927, **30**, 193.

<sup>2</sup> Thienes, C. H., and Hockett, A. J., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 793.

ter<sup>3</sup> to increase the augmentor action of tyramine. The Magnus technic with longitudinal strips and Tyrode's solution were used. The pH of the bath was 7.6, and was not altered by the aeration, nor by the drugs added. A given amount of the drug was put in the organ-bath, and, after the response had been recorded, was washed out with fresh Tyrode. Before the next application, time was allowed for complete recovery of the tissue to its initial activity. A second application of the same amount of the drug was then made, and the 2 responses were compared. In the cocaine experiments the required amounts of cocaine to give concentrations between 1:15,000 and 1:2,500,000 were added to the bath before the second application of the drug being studied, so that the second response was obtained in the presence of the cocaine. The effects of barium were studied in 66 strips, tyramine in 34, epinephrine in 42, and ephedrine in 15, the data being sufficient to give reliable averages. Analysis of the results showed that the initial tonus level, the amplitude of contractions, or the concentrations of the drugs (if submaximal) did not condition the type of response obtained after cocaine. Hence these factors need not be considered in detail. The significant results may be briefly summarized with reference to tonus changes.

*Barium:* Two successive applications of barium (1:1,700 to 1:50,000) to uncocainized strips produced equal increases of tonus, but, if the intestines were cocainized before the second application, the barium response was diminished by 37%. Using atropinized and uncocainized strips, a striking difference was seen: 2 successive applications of barium no longer produced equal responses, the second response being 193% (average) greater than the first. When such strips were cocainized before the second application of barium, the response was still increased, but to a lesser degree (43%). Under both conditions, therefore, cocaine decreased the degree of the barium-response as compared with the controls. The mechanism of the sensitization of barium to barium by atropine has not been gone into further, but would seem to merit investigation.

*Epinephrine:* When concentrations of epinephrine were used (1:500,000 to 1:50,000,000) giving small responses (up to 1 cm. inhibition), the second application, to both the cocainized and uncocainized strips, gave a slightly smaller (14%) fall of tonus. With concentrations of epinephrine causing inhibitions of tonus greater than 1 cm., in uncocainized strips, the second response was 46% smaller, and 48% smaller in the presence of cocaine. The higher concentrations of epinephrine apparently depressed the responsive-

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<sup>3</sup> Burn, J. H., and Tainter, M. L., in press.

ness of the intestines to the subsequent application of the drug. However, cocaine did not modify either the large or small responses to epinephrine, when the differences between the first and second doses were taken into consideration. This result was in contrast to the results of Burn and Tainter<sup>3</sup> using the cat intestine, where cocaine desensitized to epinephrine, but was in agreement with those of Thienes and Hockett<sup>2</sup> on rabbit and guinea pig intestines, which showed no sensitization and apparently only occasional desensitization.

*Tyramine:* Tyramine augmented 28 strips of intestine and inhibited 6 in concentrations from 1:2,500 to 1:100,000. The augmentation was not as marked as that of barium, but was definite (6 mm., average) and constant. This predominance of muscular stimulation of the rabbit's intestine by tyramine confirmed the earlier results of Tainter.<sup>4</sup> The second response to tyramine was only about one-third the first, in both cocainized and uncocainized strips. Hence, cocaine did not alter the response to tyramine. In 5 strips which were atropinized, but not cocainized, the second response to tyramine was double the first. This unusual sensitization to successive doses of the same drug in atropinized strips resembled the responses to the muscular stimulant barium, and hence, might be interpreted as further evidence of the muscular origin of the augmentor response to tyramine. Cocaine was used on only 2 such atropinized strips, but both responded with desensitization, *i. e.*, decreased response to the second application. This was also in keeping with the effects of barium on such strips.

*Ephedrine:* This drug was rather unsatisfactory because of extreme variability of response. With concentrations ranging between 1:10,000 and 1:25,000, in 11 strips, the first response was inhibitory, and in 4 it was augmentory. The second application of ephedrine produced only one-fourth the initial response, and cocainization between the 2 applications did not definitely alter this ratio.

*Conclusions:* Cocaine desensitizes excised strips of rabbit's intestine to barium, but not to epinephrine, tyramine and ephedrine. With atropinized but uncocainized strips, the second application of barium or tyramine results in marked sensitization. This sensitization is antagonized by cocainization before the second application of barium or tyramine. The failure to demonstrate the same kind of cocaine-sensitization and desensitization to these drugs in rabbit's intestinal muscle, as is seen in the muscles of the circulatory system, suggests that the mechanisms of the responses after cocaine may vary with different tissues.

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<sup>4</sup> Tainter, M. L., *J. Pharm. Exp. Therap.*, 1926, **30**, 163.

## 5450

## Bacterial Endospore Formation in Media of Varying Biologic Value.

O. B. WILLIAMS.\* (Introduced by Karl F. Meyer.)

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Although the nature of the stimulus which causes a spore forming organism to enter the spore state is not known, a number of theories have been advanced, two of which are probably the most widely cited and most readily investigated experimentally. The first of these, advanced by Buchner,<sup>1</sup> holds that the inciting stimulus is to be found in a "local" exhaustion of nutrient material in the presence of an abundance of previously well nourished cell protoplasm. Turro<sup>2</sup> ascribed the cause of spore formation to the accumulation of metabolic by-products.

Some evidence on this subject is afforded by observations made on *B. subtilis* grown in peptone water of 0.5, 1, 2, 3, 4, and 5% peptone content. Inoculations were made in duplicate from an invigorated broth culture to 50 ml. flasks containing between 10 and 15 ml. of medium at a reaction of approximately pH 6.8. A count of 1000 cells of the inoculum gave 314 spores to 686 vegetative cells. Adequate exposure to oxygen was insured by the shallow layers of the medium. Incubation was at 37°C., the optimum for the strain of *B. subtilis* used. Smears were made from each flask at daily intervals for 7 days and again at 10 days, after which the observation was discontinued. From each smear, after staining, a total of 500 cells, including both spore and vegetative forms, was counted. There was no appreciable difference between duplicate flasks in either daily counts or total counts. In only 5 instances did the daily counts fail to agree within 15% of the average, and with the total counts the agreement in half the cases was within 2% or less and in no case exceeded 7%. As a matter of convenience the values obtained have been added together for plotting. The results are given in Table I and shown graphically in Fig. 1.

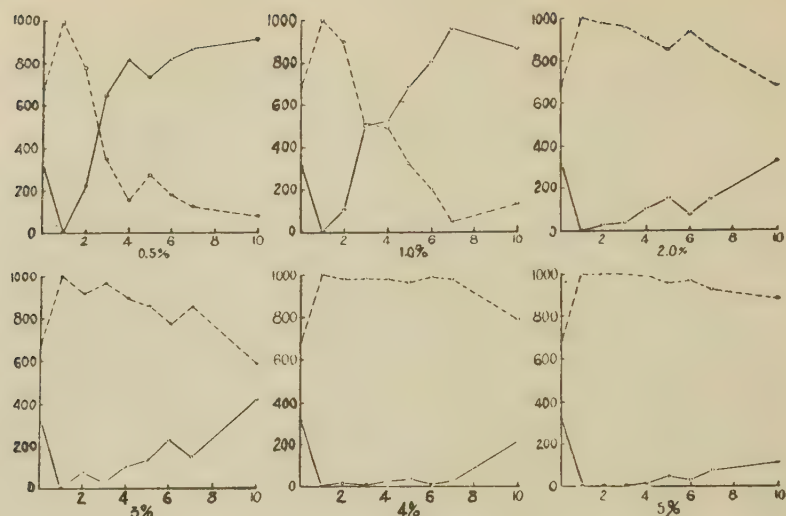
A consideration of these results at any time interval shows, with only a few minor discrepancies, that the percentage of spores varies inversely with the amount of peptone in the medium.

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\* Now at the University of Texas, Austin.

<sup>1</sup> Buchner, H., *Sitzungsberichte der Math. physik Classe der Wissenschaften zu München*, 1880, X, 368. *Centralbl. Bakt.*, 1890, **8**, 1; 1896, I, **20**, 806.

<sup>2</sup> Turro, R., *Gaceta medica catalana*, 1891, 3 and 4. (Abst. in *Centralbl. Bakt.*, 1891, **10**, 91.



Abcissa—Days. Ordinate—Cells counted.  
Broken line—Vegetative cells. Solid line—Spores.

FIG. 1.

TABLE I.

Day→	1		2		3		4		5		6		7		10	
% peptone	S	V	S	V	S	V	S	V	S	V	S	V	S	V	S	V
0.5	7	993	222	778	650	350	819	181	735	265	822	178	872	128	916	84
1	0	1000	109	891	497	503	520	480	680	320	796	204	956	44	866	134
2	0	1000	26	974	38	962	106	894	150	850	72	928	148	852	324	676
3	0	1000	80	920	33	967	104	896	133	867	227	773	144	856	419	581
4	0	1000	17	983	13	987	25	975	39	961	15	985	27	973	218	782
5	0	1000	0	1000	2	998	15	985	47	953	32	968	76	924	115	885

S = Spore. V = vegetative cell.

Determinations by direct microscopic count of the absolute number of spores and of vegetative cells in 1% and 5% peptone water after 10 days' incubation gave the values shown in Table II.

TABLE II.

	Spores $\times 10^6$	Vegetative cells $\times 10^6$	Total $\times 10^6$	Ratio—Spores to vegetative cells.
1% peptone water	952	497	1,449	1.94
5% peptone water	1,915	7,127	9,042	.27

If we assume, as would seem justifiable, that the ratio of spore to vegetative cell is of more significance in the physiology of spore formation than is the absolute number of spores formed, these re-

sults indicate that a depletion, or comparative paucity, of nutrient material is more important in promoting spore formation than is the accumulation of metabolites.

## 5451

### Effects of Certain Broncho-Constricting Drugs on Intrapleural Pressure.\*

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Report has previously been made<sup>1</sup> that following the administration to dogs of such typical broncho-dilating drugs as epinephrin and atropine, there is a definite increase in intrapleural pressure, *i. e.*, intrapleural pressure becomes more positive than is normally the case. This was interpreted as being due to lessened suction on expansion of the chest because of lessened resistance to the movement of air in and out of the lungs as a result of broncho-dilatation. It naturally became of interest to determine by direct observation whether or not the corollary is also true, *viz.*, that broncho-constriction is followed by the development of more negative intrapleural pressure than is normally present.

We employed the same dogs as had been used in our former experiments, and we had sufficient observations on normal intrapleural pressures in these animals to enable us to judge whether or not changes following drug administration were beyond normal diurnal variation. The technique used was the same as that previously described.<sup>1</sup>

Following the subcutaneous injections of solutions of pilocarpine nitrate and physostigmine (eserine) salicylate, we found uniformly in 6 experiments with each drug that intrapleural pressure definitely became less, *i. e.*, more negative. The experiments were made on 3 dogs, with intervals of a week or more between experiments. We were unable to draw definite conclusions following the administration of histamine or of arecoline because of marked disturbances in character of respiration and in the condition of the animal. Quantitative data from sample experiments may be found in Table I.

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\* Supported in part by the J. J. and Nettie Mack and the Purington Research Funds.

<sup>1</sup> Brill, S., and Leake, C. D., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 518.

TABLE I.

Dog—Wt. 18 kilos. Light sodium amytal anesthesia. Intrapleural pressure readings in left chest after subcutaneous administration of 10 mgm. eserine salicylate and of 20 mgm. pilocarpine hydrochloride on January 10 and January 20 respectively.

Time after drug.	I.P.P. in Cm. H <sub>2</sub> O after eserine.	I.P.P. in Cm. H <sub>2</sub> O after pilocarpine
Normal	—4.0 to —7.0	—4.0 to —6.4
5 minutes	—4.0 to —8.4	—3.4 to —6.2
10 "	—2.6 to —11.6	—3.5 to —7.0
15 "	+1.4 to —21.0	—5.2 to —10.8

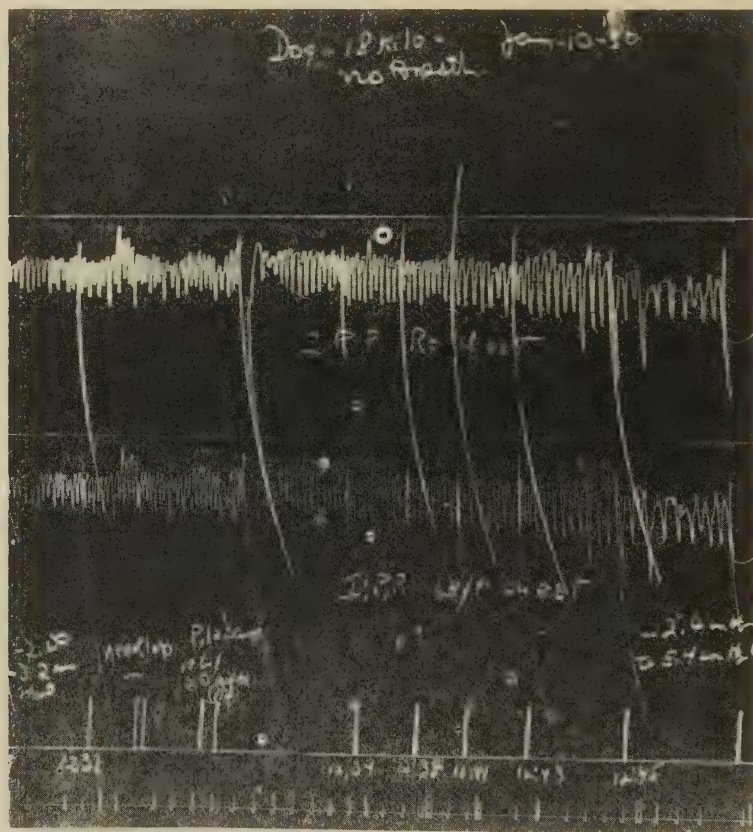


FIG. 1.

Kymographic record showing effects of pilocarpine on intrapleural pressure in a dog.

The factors involved in the development of more negative intrapleural pressure following broncho-constriction are not as easy to evaluate as might be expected. With broncho-constriction greater resistance is offered to the passage of air in and out of the alveoli

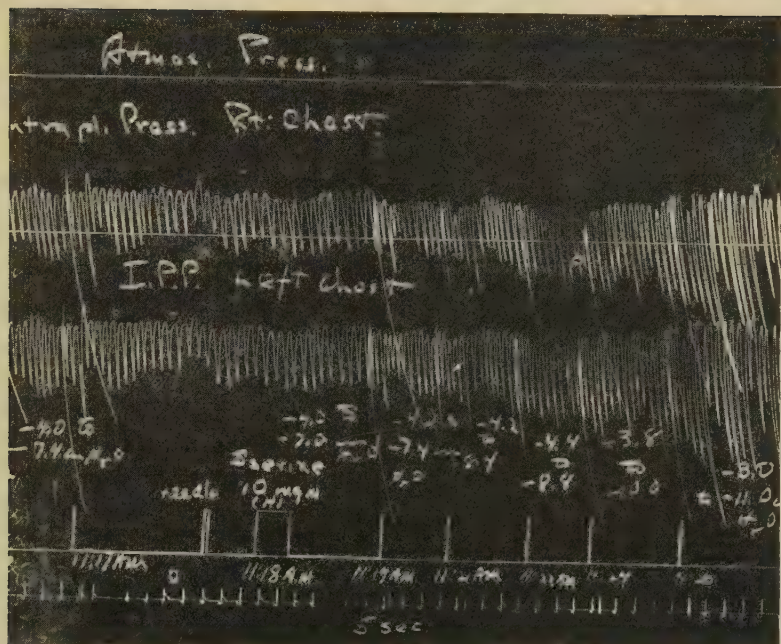


FIG. 2.

Kymographic record showing effects of eserine on intrapleural pressure in a dog.

than is ordinarily the case. With usual inspiratory effort one would expect then that more suction is developed on expanding the chest than normally, and that this should be reflected in a more negative intrapleural pressure than normal. But on expiration one would expect the reverse to occur, *i. e.*, that more pressure would be developed on relaxation of the chest, or in active expiratory effort, and that this would be reflected in more positive intrapleural pressure than normal. While we observed, with broncho-constriction caused by pilocarpine or eserine, the development of a more positive intrapleural pressure on expiration, it was by no means proportional to the development of a more negative intrapleural pressure on inspiration. This suggests the operation of some other factor. Resistance to the passage of air in and out of alveoli may be expected to be accompanied by a gradual increase of carbon dioxide tension in the alveoli and blood. Davies, Haldane and Priestley<sup>2</sup> showed that cotton-wool resistance to nasal breathing definitely increased alveolar carbon dioxide percentage, and Meakins<sup>3</sup> found carbon

<sup>2</sup> Davies, H. W., Haldane, J. S., and Priestley, J. G., *J. Physiol.*, 1919, **53**, 60.

<sup>3</sup> Meakins, J. C., and Davies, H. W., *Respiratory Function in Disease*, Edinburgh, 1925, 195.

dioxide retention in paroxysmal asthma. We thought it expedient, therefore, to investigate the effect of direct alterations of alveolar carbon dioxide tensions on intrapleural pressure, and our observations in this regard will be presented later.

## Southern Section.

Tulane University, February 14, 1931.

5452

### Habitat of *Giardia* in the Intestine.

ERNEST CARROLL FAUST.

*From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University.*

Authorities on intestinal protozoa (Hegner,<sup>1</sup> Wenyon,<sup>2</sup> Lynch<sup>3</sup>) state that *Giardia* lives in the upper part of the small intestine, mainly in the duodenum rather than in the cecum which is a common habitat for *Trichomonas* and *Chilomastix*. Hegner<sup>4</sup> has described *Giardia canis* as the species present in the dog, but has not recorded the level of the intestine in which this organism has been found. In connection with our Tulane Strain A of *Endamoeba histolytica* in dogs we have encountered *Giardia canis* as a contamination. In maintaining the ameba *in vivo* by continuously subinoculating one dog from another *per rectum* (Faust), we have had an opportunity to study in life and at autopsy dogs which have received the *Giardia* as an experimental infection. These observations we have compared with data obtained from naturally infected dogs.

In the majority of our cases harboring a natural *Giardia* infection the primary seat of the organism is in the cecum and appendix; frequently it is also in the colon and the rectum; occasionally it is found in the distal 10 cm. of the ileum; it has never been seen by us in the higher levels of the small intestine. In experimental infections of our dogs, previously found by repeated fecal examination to be negative for intestinal protozoa, we have encountered a somewhat different situation. The active *Giardia* trophozoites were in-

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<sup>1</sup> Hegner, R. W., Animal Parasitology, by Hegner, Root and Augustine, 1929, 119.

<sup>2</sup> Wenyon, C. M., *Protozool.*, 1926, **1**, 702.

<sup>3</sup> Lynch, K. M., Protozoan Parasitism of the Alimentary Tract, 1930, 187.

<sup>4</sup> Hegner, R. W., *Am. J. Hyg.*, 1922, **2**, 448.

roduced by rectal tube into the upper levels of the large bowel and usually expelled there. In some cases the tube passed the ileo-cecal valve and the inoculum was discharged anterior to the valve. In nearly 50 cases available for analysis an infection of 100% has been obtained. In most of the animals the active *Giardias* were consistently present in the feces from the second day; in 4 instances they

Autopsy findings in dogs previously negative for intestinal protozoa, experimentally infected with *Giardia canis*.  
TABLE I.

Dog No.	Incuba- tion period	Death in days af- ter inoc- ulation	Motile <i>Giardias</i> found in							
			rectum	colon	cecum	appen- dix	post. ileum	ant. ileum	jejunum	duode- num
1	2 days	14 days	+	+	+	+	+	+	-	+
2	2 "	14 "	+	+	+	+	+	+	-	-
3	2 "	32 "	+	+	+	+	+	-	-	-
4	1 "	55 "	-	-	-	-	-	-	-	-
5	1 "	27 "	+	-	+	+	+	+	+	+
6	1 "	18 "	+	-	+	+	+	+	+	+
7	3 "	50 "	+	-	+	+	+	+	-	-
8	2 "	20 "	+	+	+	+	-	-	-	-
9	3 "	17 "	+	+	+	+	-	-	-	-
10	1 "	15 "	+	+	+	-	+	-	-	-
11	7 "	21 "	-	-	-	+	-	-	-	-

were recorded as early as 24 hours after inoculation. In 11 of these cases autopsies were performed immediately *post mortem*, while the animals were still warm. The autopsy findings are recorded in Table I.

The data demonstrate the preponderance of foci in the cecum, appendix and rectum. However, in 7 cases the organisms were found in the lower ileum; in 3 cases in the upper ileum, in one case in the jejunum, and twice in the duodenum. In the 2 instances in which colonies were found in the duodenum (in one case in and around the ampulla of Vater) we may conclude that the organisms migrated to this level against the peristaltic waves of the intestine, since only trophozoites were passed in the stools, thus eliminating the possibility of oral infection from ingestion of cysts.

### 5453

#### Effect of Carbon Dioxide on Ether Anesthesia.

GEORGE B. KLEINDORFER. (Introduced by J. T. Halsey.)

*From the Department of Pharmacology, Tulane University.*

In anesthesia, more especially where there is any significant amount of rebreathing, it is evident that the patient is inhaling a gas mixture containing considerable amounts of  $\text{CO}_2$ . As was established long ago and as has again been shown by Leake and Waters,<sup>1</sup> 30 to 40% of  $\text{CO}_2$  has distinct anesthetic properties. It, therefore, seems possible that in anesthesia where the patient is breathing fairly high percentages of  $\text{CO}_2$ , this gas may be acting synergistically or additively with the anesthetic given, and exerting an appreciable anesthetic action. It also seems reasonable that if such be the case,  $\text{CO}_2$  might be administered simultaneously with other anesthetics with the object not only of stimulating the respiration but also to act as an adjuvant to them.

To test these views we have studied the effect of  $\text{CO}_2$  on ether anesthesia in some 50 experiments on 11 full grown white rats with the following results:

Moderately deep anesthesia\* almost invariably resulted from the

<sup>1</sup> Leake, C. D., and Waters, R. M., *Anesthesia and Analgesia*, Feb., 1929, **8**, 17.

\* The criterion for depth of anesthesia was the response in the fore legs and the neck to stimulation of the hind legs by alternating current with secondary coil at different distances. Response to the stimulation by squealing was also used but found to be very irregular and consequently less reliable.

inhalation for 30 minutes of air containing 3.5 to 3.8% ether and  $\text{CO}_2$  of from 1 to 2% (arising from the rat's metabolism). Less than 3.5% of ether with similar low percentages of  $\text{CO}_2$  caused incomplete anesthesia, except that, in one experiment, fairly complete anesthesia was obtained with 3.32% ether with 1%  $\text{CO}_2$ . Attempts to obtain a like result with similar percentages in the same rat and in several others failed in any of 5 such experiments.

Inhalation for a similar period of from 2.2 to 2.8% ether and from 5.1 to 12%  $\text{CO}_2$  resulted in 15 out of 21 experiments in an anesthesia equal in depth to that from the higher ether and low  $\text{CO}_2$  concentration. In 3 of this series only a moderately deep anesthesia was obtained, the mixture inhaled being ether 2.45, 2.09 and 2.26%, with  $\text{CO}_2$  9.4, 8.04, 7.08%. In 3 others with ether 2.1, 2.31, 2.12% and  $\text{CO}_2$  9.92, 12.09, 6.4% a hardly appreciable degree of anesthesia resulted. In contrast to such incomplete anesthesia with the above concentrations there may be cited experiments in which good anesthesia was obtained with ether 2.02, 2.24, 2.36 and  $\text{CO}_2$  8.25, 7.85, 7.75%. In no case did 2.5% or more of ether with  $\text{CO}_2$  percentages ranging from 6 to 10% fail to induce anesthesia equal to that produced by 3.5 to 3.8% ether. With ether concentrations of less than 2% only a very moderate degree of anesthesia resulted even with as much as 12% of  $\text{CO}_2$ .

Observations made on 2 rabbits and one cat were in accord with Leake's findings as to the apparent harmlessness of the inhalation of such concentration of  $\text{CO}_2$ , as in these experiments a switch from ether about 3.7% to low ether and high  $\text{CO}_2$  concentrations regularly resulted in a rise of blood pressure and apparent improvement in the circulation, especially if it had been impaired prior to this switch.

*Conclusions.* In white rats 60 to 80% of the anesthetic concentration of ether when inhaled with 16 to 40% of the anesthetic concentration of  $\text{CO}_2$  can produce an anesthesia equal in depth to that resulting from the inhalation of ether in anesthetic concentration (3.5 to 3.8%).

A study of the effect of  $\text{CO}_2$  on anesthesia with ethylene and nitrous oxide is in progress.

5454

### Correlation of Uric Acid Production with Growth of Kidney Tubules in Chick Embryos.

EDWARD A. BOYDEN.

*From the Department of Anatomy, University of Alabama.*

In previous microchemical studies it was shown<sup>1</sup> that uric acid is the chief end-product of nitrogen metabolism in chick embryos, that it begins to accumulate in the allantoic cavity sometime during the fifth day of incubation and that the rate of its excretion rises, in general, proportionally with the body weight. By the end of the first 13 days of incubation the allantoic fluid contains, on the average, 15 mg. of uric acid dissolved more or less completely in about 6 cc. of water. These facts have sufficed to prove that the mesonephros is an active excretory organ and that protein is burned by the developing embryo to a significant degree.

The present article represents a continuation of this cooperative study with Dr. Fiske, in which attention has been focused on the morphological development of the mesonephric tubules. As a basis of study 7 serially sectioned chick embryos from the Harvard Embryological Collection, ranging from 5 to 14 days of incubation, were selected for reconstruction. These were graded embryos that had been collected and serially sectioned, some years before, under the supervision of the writer. Every third section throughout the length of the mesonephros of each embryo was drawn to scale under an Edinger projection apparatus. On these drawings each glomerulus was systematically recorded and the total number counted. Then, by the Born method, wax models of the Wolffian bodies were reconstructed. Finally, at a much higher magnification, 7 mesonephric tubules—one from each embryo, and selected from comparable portions of the left Wolffian body—were reconstructed in wax. The modelling of these tubules proved to be a difficult undertaking, especially in the 55 mm. embryo where the convolutions were exceedingly tortuous. After the models were completed, the total volume of each was obtained by submerging it in water and measuring the displaced fluid. Then each model was divided into glomerular, secreting and collecting portions, and the volumes of the 3 parts separately measured.

By these methods it was ascertained that in the period ranging from 5 to 14.2 days of incubation the Wolffian body (including both vascular and nephrogenic tissue) increases its volume approximately

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<sup>1</sup> Fiske and Boyden, *J. Biol. Chem.*, 1926, **70**, 535.

12 times, although the total glomerular count shows no significant increase. During the same period representative tubules increase their volume approximately 16 times. The most striking feature, however, is the inequalities of growth manifested by different parts of the mesonephric tubule. Thus, while the renal corpuscle is increasing its volume approximately 3 times, and that of the collecting portion of the tubule about 10 times, the secretory tubule increases its volume about 33 times.

As a check on the growth of the renal corpuscle 6 glomeruli from each embryo were separately modelled and their volumes ascertained. These figures show that although the glomerular knot, as contrasted with the whole renal corpuscle, is becoming more lobulated, it increases in volume only  $2\frac{1}{2}$  times during the period from 5 to 14.2 days of incubation. Incidentally it is of interest to note that the mesonephric glomerulus of the 14.2 day chick is about 50 times the size of a glomerulus from the metanephros of the same embryo. (The tubular portions were too small to model.)

Similarly, linear measurements of the mesonephric tubules indicate that the secretory portion of the tubule is the part that is elongating most rapidly. Thus, from 5 to 14.2 days of incubation—when the whole mesonephric tubule (including the glomerulus) is increasing in length approximately  $6\frac{1}{3}$  times, and its collecting portion about  $3\frac{1}{2}$  times—the secretory portion increases 9 times in length.

From a preliminary analysis of this data, therefore, it is apparent that during the period when uric acid is being increasingly excreted, the secreting portion of the mesonephric tubule is the part that is growing the fastest.

## 5455

### Developmental Potencies of Explanted Quadrants of Hensen's Node.

THOMAS E. HUNT. (Introduced by E. A. Boyden.)

*From the Department of Anatomy, University of Alabama.*

This article is part of a series of studies designed to test the potencies of the embryonic chick blastoderm, particularly that part of the primitive streak designated as Hensen's node. When this node, in stages prior to the formation of the head-process, is transplanted to the chorio-allantoic membrane of 9-day chicks, the grafts

that develop contain organs and tissues that are found in the normal embryo as far back as the mesonephros, including tubules (and glomeruli) from that organ. From these observations previously reported<sup>1</sup> it was concluded that Hensen's node at this stage is essentially totipotent. It was also shown that other parts of the blastoderm have a limited capacity, as indicated by the fact that grafts of the area pellucida anterior to the node, or the primitive streak posterior to it, form generalized structures only, such as gut, cartilage, muscle and skin with feather germs.

The present experiments were undertaken to ascertain the potencies of different parts of the node. As before, donor blastoderms were used in which there was a definitive primitive streak but no head-process. A median longitudinal cut was made through the primitive streak and a transverse cut through the primitive pit, as a consequence of which the node and area pellucida were divided into 2 equal anterior parts and 2 equal posterior parts. As it was easier to handle large pieces and as regions outside of the node have limited capacities, the area pellucida in the majority of transplants was not excluded. In the remaining experiments only that part of the area pellucida immediately surrounding the node was included with the nodal tissue. As both types of experiment yielded the same results they are considered together.

Of the grafts obtained, 68 have been studied and form the basis of this preliminary report. Twenty of these were obtained from the left anterior, 22 from the right anterior, 14 from the left posterior and 12 from the right posterior, quadrant. No experiment was considered valid unless grafts were obtained from at least 2 adjoining quadrants of the node. Out of 28 experiments thus retained, 19 gave grafts from 2 quadrants, 6 from 3 quadrants and 3 from all 4 quadrants.

An inventory of the tissues found in these grafts indicates that the right and left anterior quadrants are equipotent. In such grafts from adjoining portions of the same donor, vesicles of the fore-, mid- and hind-brain were clearly differentiated. In many cases these were comparable in size and histological appearance to portions of the brain derived from grafts of the entire node. Closely associated with the brain, in 78% of the cases, was notochordal tissue. Besides brain and notochord the following organs developed in grafts from the anterior quadrants of the same donor; eye, ear, epiphysis, hypophysis, heart, liver and gut. In addition to these

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<sup>1</sup> Hunt, T. E., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 84, 86; *J. Exp. Zool.*, 1931, **59**, No. 3 (in press).

there were such common tissues as cartilage, skeletal muscle, dermis, epidermis and feather germs. Thyroid occurred in grafts from either the right or left quadrant but not as yet from both quadrants of the same donor.

Similarly, right and left posterior quadrants appear to be essentially equipotent. The parts of the neural tube found in grafts from these regions were hind-brain and spinal cord. A well-formed tube or vesicle occurred less frequently than in the anterior halves and the nervous tissue was often disorganized. Notochord was associated with the nervous tissue in 53% of the cases. Heart, liver, mesonephros and the common tissues mentioned above were also found. Occasionally suprarenal gland occurred in grafts of one quadrant or the other.

In contrast to the equipotency of the right and left sides, there is a dissimilarity in the potencies of the anterior and posterior halves of Hensen's node. When either the 2 right or the 2 left quadrants were grafted on the chorio-allantoic membrane, it was found that the anterior quadrants had the capacity to form such structures as brain, eye, epiphysis and hypophysis; whereas the posterior ones gave rise to spinal cord (and possibly hind-brain), mesonephros and suprarenal gland. All 4 quadrants produced notochord and the usual tissues. It is believed that heart and liver may also be formed in all 4 areas but thus far they have been found in only 3 of the quadrants from the same donor.

In conclusion, it is evident that right and left anterior quadrants are equipotent, that the same holds true for right and left posterior quadrants but that the anterior and posterior halves of Hensen's node differ in their capacities.

## 5456

### Changes in the Composition of the Red Blood Corpuscle During Fat Absorption.

M. BODANSKY.

*From the John Sealy Memorial Research Laboratory and the University of Texas School of Medicine.*

The composition of the red blood corpuscle may be altered physiologically by the simple expedient of feeding a fat, such as olive oil. The changes thus produced have been studied by Bloor,<sup>1</sup>

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<sup>1</sup> Bloor, W. R., *Physiol. Rev.*, 1922, **2**, 92.

Knudson<sup>2</sup> and others and it has been determined that during fat absorption there is an increase in the lecithin content of the blood which runs more or less parallel to the change in total fatty acids, the increase being more marked in the corpuscles than in the plasma. As regards cholesterol, Knudson has shown that the esters of this substance increase in amount during fat absorption, particularly in the corpuscles, where normally little or no cholesterol is present in the ester form.

These changes have been considered of significance chiefly from the standpoint of fat transport and metabolism and the suggestion has been made that the red blood cells absorb the fat from the

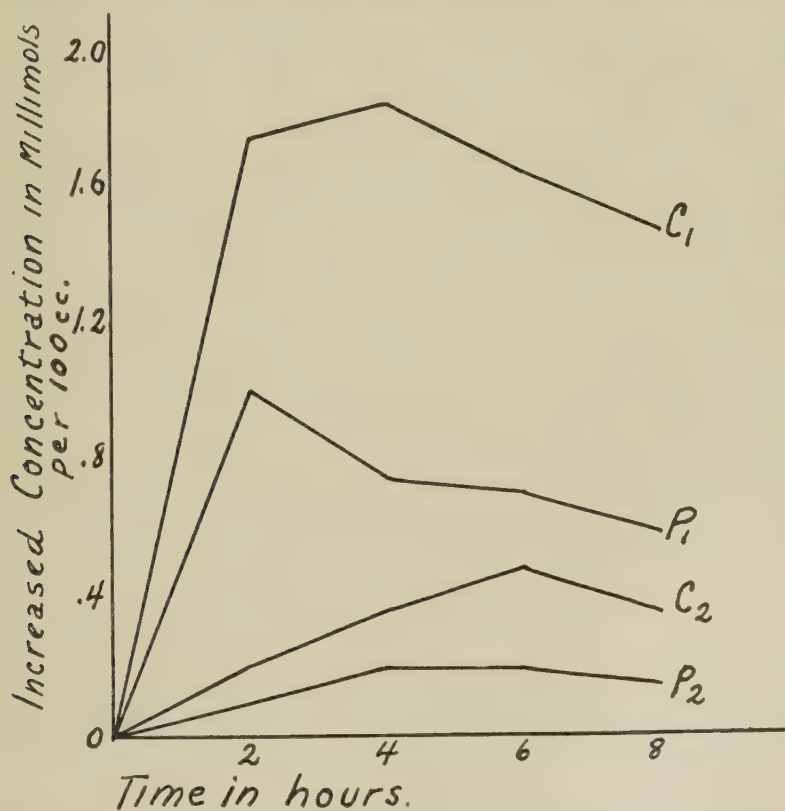


FIG. 1.

Effect of ingestion of olive oil in increasing the concentration of total fatty acids and of fatty acids in combination as phosphatide and cholesterol esters.  $C_1$  = total fatty acid increase in the corpuscles;  $P_1$  = total fatty acid increase in the plasma;  $C_2$  = increased concentration of lecithin and cholesterol esters in the corpuscles;  $P_2$  = increased concentration of lecithin and cholesterol esters in the plasma.

<sup>2</sup> Knudson, A., *J. Biol. Chem.*, 1917, **32**, 337.

plasma, transform it into lecithin and that thus the red blood corpuscles play a part in the transportation and metabolism of fat. Our interest in the problem has been in relation to the alterations in permeability of the red blood corpuscle accompanying changes in the composition of its lipid constituents. The frequently cited data of Knudson obtained in his study of fat absorption of dogs are of unquestioned accuracy, but their physiological significance may be made more apparent if represented in terms of molecular equivalents. At any time during absorption, the millimolar increase of cholesterol, present as esters, plus  $2 \times$  the millimolar increase of lecithin, obviously represent the increase, in millimols, of the fatty acid present in combination with cholesterol and as phosphatide. The remainder may be supposed to exist almost entirely as neutral fat and soap.

Figure 1 represents the data, based on these calculations, obtained in an experiment on a dog, weighing 7.5 kg., following the administration of 50 g. of olive oil. Knudson's data have been recalculated on the same basis and show close agreement to our own results. It therefore does not seem necessary to present all of our data in detail.

*Conclusions.*—During the absorption of fat the maximum increase in total fatty acid in the red blood corpuscle is usually observed at the end of 2 to 4 hours. The increase of the combined fatty acids is somewhat delayed, the maximum being noted usually at the end of 6 hours.

Though occasionally the increase in cholesterol esters and lecithin in the corpuscle is more marked, as a rule it represents less than 20% of the total increase in fatty acids.

## 5457

### Distribution of Unsaturated Fatty Acids in the Blood During Fat Absorption.

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*From the John Sealy Memorial Research Laboratory and the University of Texas School of Medicine.*

This paper considers the effect of the ingestion of olive oil on the distribution of unsaturated fatty acids in the plasma and corpuscles. Dogs were used as the experimental animals. After a 12 hour period of fasting, a specimen of blood was taken for analysis. A

definite amount of olive oil was then given by stomach tube and additional specimens of blood were collected at the end of 2, 4 and 6 hours. Analyses were made of the plasma, as well as of the whole blood. These were treated with the alcohol-ether mixture, according to the well-known method of Bloor. To insure complete extraction of the lipids from the corpuscles, the extractions were repeated several times. The iodine number of the extracted lipids was determined by a micro-modification of Hanus' method, essentially as described by Gibson and Howard.<sup>1</sup> This procedure has been found to give reliable results both in previous work<sup>2</sup> and in the present experiments.

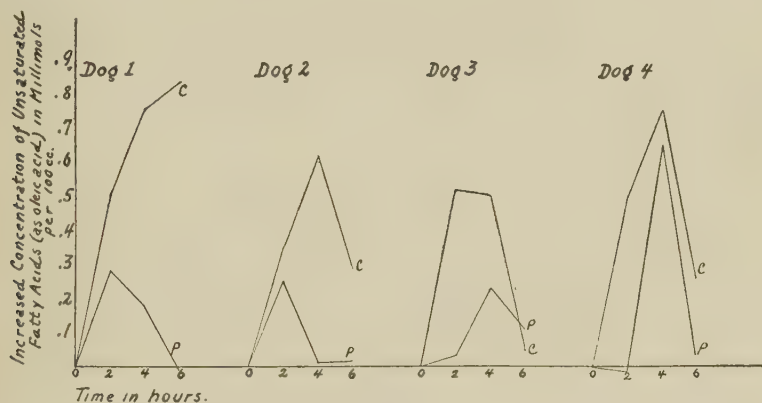


FIG. 1.

The results are represented by the curves in the accompanying figure. Dog 1, weighing 7.3 kg., received 50 cc. of olive oil; Dog 2, weighing 15.6 kg., received 65 cc.; Dog 3, weighing 11.1 kg., received 60 cc.; Dog 4, weighing 7.5 kg., received 50 cc. As shown by the curves, the increase in concentration of unsaturated fatty acids was always greater in the corpuscles (C) than in the plasma (P). The increase, however, was very small considering the amounts of fat administered. Taking Dog 4 as an example, the volume of blood, assuming it to be approximately 8.5% of the body weight, was about 635 cc. The maximum increase in the concentration of the unsaturated fatty acids was observed in this case at the end of 4 hours after administration and amounted to 0.7 millimols of oleic acid per 100 cc. of whole blood. Accordingly, at this time, there was in circulation 4.45 millimols, or 1.25 g. of oleic acid more than at the beginning of the experiment. This was only about

<sup>1</sup> Gibson, R. B., and Howard, C. P., *Arch. Int. Med.*, 1923, **32**, 1.

<sup>2</sup> Bodansky, M., *J. Biol. Chem.*, 1925, **63**, 239.

one-half of the increase in total fatty acids, which at the end of 4 hours amounted to 1.27 millimols per 100 cc. of whole blood, or 2.3 g. of fatty acid (in terms of oleic acid). Considering the red blood corpuscles alone, the average maximum increase of the unsaturated fatty acids was .68 millimols, which is only about one-third of the total fatty acid increase (average of 1.92 millimols).

It is difficult to explain why after the administration of olive oil the increase in total and saturated fatty acids, particularly in the corpuscles, should be greater than the increase in oleic acid. This observation, first made by the author in 1924, is in agreement with a similar finding of McClure and Huntsinger<sup>3</sup> that the increased concentration of lipids in the blood which follows the absorption of oleic acid is not solely the result of accumulation of this substance. Either the oleic acid undergoes saturation, or what is more likely, saturated fat is mobilized from the fat depots during fat absorption.

## 5458

Relation of Hemolysis to the Primary Penetration of Fatty Acids  
Through the Red Cell Membrane.

M. BODANSKY.

*From the John Sealy Memorial Research Laboratory and the University of Texas  
School of Medicine.*

The hemolytic action of fatty acids may be attributed to the combined effects of injury of the erythrocyte at its surface and secondary penetration. It is obvious, however, that in high concentrations of the hemolytic agent, the damage to the membrane may in itself be sufficient to cause the dissolution of the cell, in which case the factor of permeability is of negligible importance. On the other hand, if the concentration is sufficiently low, the change in the red blood cell membrane may be so slight that a condition is approached in which primary penetration of the fatty acid may be regarded as the sole factor involved in the process.

That under certain conditions measurement of the rate of hemolysis also determines the rate of permeability of the hemolytic agent has been considered elsewhere<sup>1</sup> and is supported by the observation that the hemolytic action of fatty acids parallels their behavior in a

<sup>3</sup> McClure, C. W., and Huntsinger, M. E., *J. Biol. Chem.*, 1928, **76**, 1.

<sup>1</sup> Bodansky, M., *J. Biol. Chem.*, 1928, **79**, 241.

wide variety of biological phenomena in which primary penetration of these acids into tissues is involved.

Another test of the osmotic nature of the process may be made by varying the tonicity of the hemolytic system. It has been shown previously<sup>2</sup> that increasing the osmotic concentration of the fluid outside the corpuscles has little effect in altering their resistance to the hemolytic action of inorganic acids, which makes it seem probable that injury to the membrane is the primary factor involved. On the contrary, alterations in the concentration of the outside fluid exert a marked effect in hemolysis by fatty acids, which indicates that here penetration into the corpuscle is the predominant factor. This is illustrated in the accompanying chart, which represents the relation of tonicity (expressed in per cent NaCl) to the resistance of dog's corpuscles to the hemolytic action of fatty acids.

For a given tonicity, the ratio  $\frac{\text{time required for complete hemolysis}}{\text{time required for complete hemolysis in a system isotonic with 0.85\% NaCl}}$  has been taken to represent the resistance. The circles represent data obtained in systems containing 0.04 N butyric acid; the squares, data obtained with 0.02 N valeric acid; the triangles, the data with 0.008 N caproic acid.

As is evident from the chart, the hemolytic effect is profoundly modified by changes in the osmotic concentration of the outside fluid. Thus, in a system equivalent to 0.68% NaCl (0.8 isotonic), the resistance of the erythrocytes to fatty acids is only about half the resistance in an isotonic solution. In a system equivalent to 1.2% NaCl, the resistance is increased approximately 50%.

Because of the independent effect of sodium chloride on erythrocytes, the data obtained in strongly hypertonic systems are not included in the present series of experiments. To illustrate the effect of exposure to hypertonic saline, the following experiments may be described.

Exp. 1. Cells were suspended in 2 x isotonic saline for 5 minutes, then washed and suspended in isotonic saline. The value of R (resistance) of the resulting suspension was 0.95. The control suspension was prepared by treating cells similarly for the same period of time, but with isotonic saline only.

Exp. 2. Cells were suspended for 2-3 minutes in 5 x isotonic saline, then washed and resuspended in isotonic saline. The resistance of these cells was found to be 0.756 to caproic acid (0.008 N) and 0.744 to valeric acid (0.02 N).

It should be noted that the technique employed in the present

<sup>2</sup> Bodansky, M., *J. Biol. Chem.*, 1928, **79**, 229.

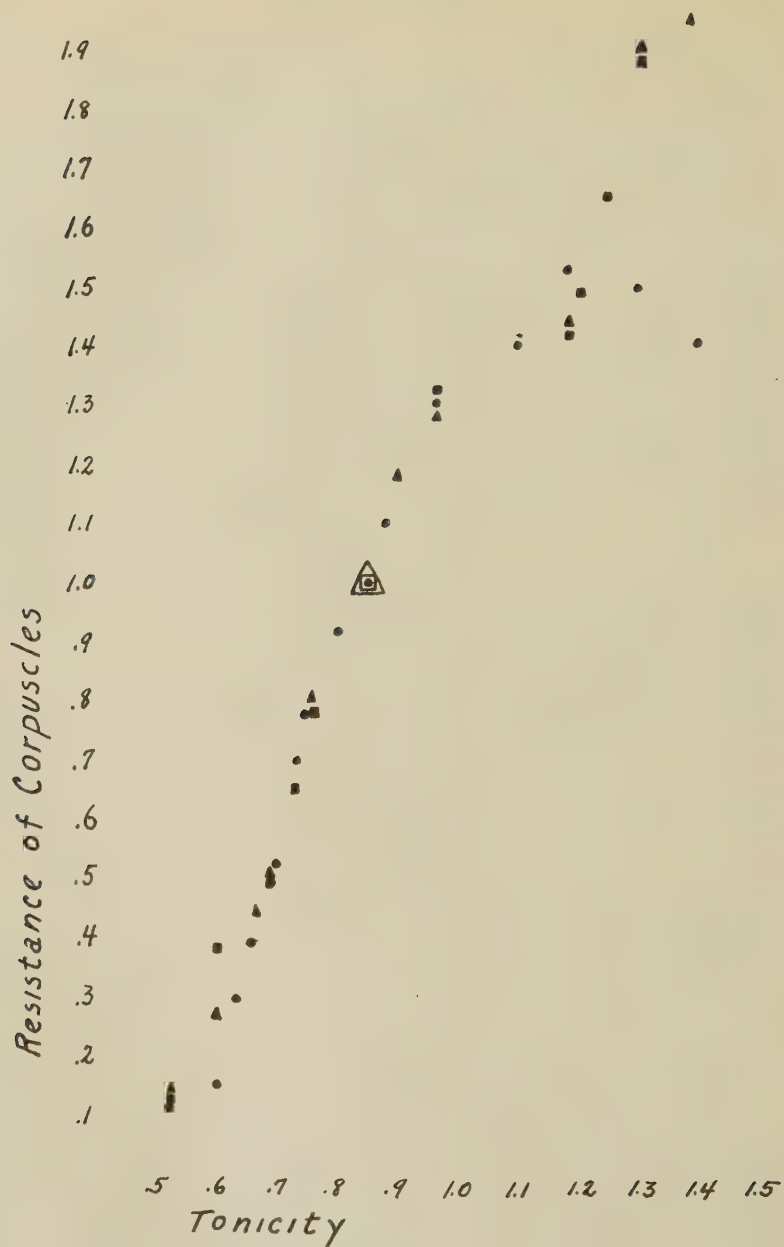


FIG. 1.

study is similar to that used in earlier work<sup>1, 2, 3</sup> and is based on methods originally devised by Ponder.<sup>4</sup>

<sup>3</sup> Bodansky, M., *J. Biol. Chem.*, 1929, **82**, 567.

<sup>4</sup> Ponder, E., *Proc. Roy. Soc. London, Series B*, 1921, **92**, 285.

### Interruption of Perfusion of Isolated Rabbit Heart upon Reaction of Coronary Flow.

W. T. DAWSON AND OSCAR BODANSKY. (Introduced by M. Bodansky.)

*From the Laboratory of Pharmacology, University of Texas School of Medicine.*

We have not found described the effect of interruption of the perfusion of the isolated mammalian heart upon the reaction of the coronary flow immediately subsequent to resumption. This very simple experiment may be used to demonstrate one of the most fundamental chemical properties of muscle, increased acid production under conditions of inadequate oxygen supply.

This increased acidity is demonstrable with the isolated rabbit heart, perfused with Ringer-Locke<sup>1</sup> solution. In some instances we have altered the formula, using  $\text{CaCl}_2$ , 0.012% instead of 0.024%, or varying the  $\text{NaHCO}_3$  strength; the former change appears immaterial to the result, but reduction in  $\text{NaHCO}_3$  strength makes the reaction very distinct.

In order that the perfusion fluid may wash the heart of blood and clear the tissue of accumulated diffusible acid products in unusual amount, perfusion is carried out for 20 or 30 minutes, when the beat has usually become fairly regular, and the fluid escaping from the heart nearly constant in reaction from minute to minute. The perfusion is then stopped by clamping off the tube leading from the Ringer reservoir, or by lowering of the perfusion pressure from the usual adequate value of 50 to 70 cm. of Ringer to about 4 cm. The latter procedure may readily be carried out with the apparatus used by us<sup>2</sup> by providing a rubber tube connection of adequate length for the Ringer reservoir.

With zero or inadequate perfusion pressure so caused, the heart not infrequently shows at first a period of increased force of beat, but soon becomes irregular and finally stops at the end of usually 8 to 20 minutes. If the perfusion is now resumed with the former pressure, the fluid escaping from the heart is more acid than before for a period of 1 to 5 minutes or even longer, and not infrequently also temporarily increased in amount.

The temporary pH change may be made as great as 2.00 re-

<sup>1</sup> Locke, F. S., and Rosenheim, O., *J. Physiol.*, 1907, **36**, 204.

<sup>2</sup> Dawson, W. T., *J. Lab. and Clin. Med.*, 1925, **10**, 853. Liddell, E. G. T., and Sherrington, Sir Charles, *Practical Mammalian Physiology*, Clarendon Press, Oxford, 1929.

duction by using about 0.004%  $\text{NaHCO}_3$  in the Ringer; with ordinary concentrations (about 0.015%) it is about 0.40 and readily seen with phenol red or bromthymol blue as indicator. The temporary increase in coronary flow may be as great as 100%, or more, and may be very constantly obtained by using an arbitrary 6 minute interruption of perfusion. The perfusate is best collected at one minute intervals, and the pH determined at once. Brown's<sup>3</sup> method is convenient.

## 5460

## Iron Deficiency in the White Rat and the White Mouse.

SIDNEY BLISS AND M. L. THOMASON.

*From the Biochemical Laboratory, Tulane University School of Medicine.*

This work is a development of the view that pellagra may be an iron-deficiency disease.<sup>1</sup> We have studied the physiological effects of iron restriction in white rats and white mice. The diets low in iron have been of 2 types: (a) A synthetic diet of casein, sucrose, butter fat, lard, a salt mixture which is iron-poor but assumed to be otherwise balanced,<sup>2</sup> vitamin B concentrate (Harris) and viosterol. (b) A diet of natural foodstuffs such as banana, orange juice, sucrose, freshly separated egg white, cream, and a salt mixture very low in iron but assumed to be balanced for the other elements, viosterol, vitamin B concentrate (Harris) and agar. These diets furnish protein of good quality in adequate amount, sufficient fat and carbohydrate and an abundance of vitamins A, B, D and G. All of our rats grew well.

Six series of from 6 to 8 rats each, and one series of 10 mice, raised in the laboratory and appearing vigorous and healthy at weaning age—about 21 days—were used. The young rats weighed at least 35 gm.

The results obtained have been entirely uniform, all rats and mice kept on these iron-poor diets developed the following symptoms:

If the young rats have not had access to rich sources of iron between the time that they open their eyes and the time of starting the experiment, the first symptoms of falling hair may appear as

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<sup>3</sup> Brown, J. Howard, *J. Lab. and Clin. Med.*, 1924, **9**, 239.

<sup>1</sup> Bliss, S., *Science*, 1930, **72**, 577.

<sup>2</sup> Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 725.

early as the 15th day of the experiment, or as late as 2 months.

The loss of hair is always strikingly bi-laterally symmetrical. The hair is not chewed off by the other rats, necessarily, because (a) when rats are kept alone the hair loss occurs in regions that are inaccessible to the rat itself, and (b) the intact hair with the bulbous enlargement, where it had been attached in the follicle, is found on the screen below the floor of the cage. Coprophagy is an important consideration in experiments of this type, and our attempts to prevent it have met with varying degrees of success.

When the daily collection of feces and cleaning of the cage reveals a layer of hair on the screen below, one observes symmetrical areas of thinning of the coat. This often begins just above the shoulders but may also occur as a streak in the exact middle of the back—or it may take the form of longitudinal areas along both sides of the rat, leaving the area over the middle of the back entirely unaffected. When the hair is lost along both sides of the rat these areas frequently extend out upon the face. Occasionally a baldness of the head develops and may spread down over the face.

The condition in rats (unlike that in another species to be described) is usually non-fatal. The denudation may become very extensive, and a dermatitis develops in the denuded areas. This involvement of the skin itself assumes a buff-colored appearance and goes through a process of scaling off. Scaling occurs at the corners of the mouth as well.

Analyses of food and feces of rats on a low-iron diet show that they are in a negative balance. The addition of ferric citrate to the drinking water or the addition of solid ferric citrate to the food has no demonstrable effect upon the symptoms over a period of months. When excess ferric citrate is eaten, diarrhea and death supervene without any alteration or alleviation of the skin or hair condition. Prompt growth of hair and return of well-being in the rat is accomplished by feeding either cooked or dried liver, dried whole dog blood or crystalline hemin prepared from dog blood or Armour's dried hemoglobin. These substances are fed in such quantities as to furnish equivalent amounts of iron, the amount given being calculated from the state of the negative balance. The kind of iron given is obviously of much importance.

This relationship between diets deficient in iron and the loss of hair is particularly interesting in connection with analyses reported for beef hair in which it was shown that gram for gram, hair contains more iron than does any other tissue, including liver.<sup>3</sup>

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<sup>3</sup> Elvehjem, C. A., and Peterson, W. H., *J. Biol. Chem.*, 1927, **74**, 433.

In the series of white mice we have tested prophylaxis with crystalline hemin. Ten white mice from 3 litters were divided into 2 groups of 5 each—weighing 42 gm. per group at weaning age—21 days. Both groups were fed diet B. One group received a solution of crystalline hemin as an addition to the drinking water. Within 19 days all 5 mice on the iron-poor diet had developed symptoms as described above for rats, while the group which was protected by the addition of hemin was absolutely untouched by any of the symptoms at the time of writing (30th day).

Since our rats receive an abundance of the complete vitamin B group of substances, yet show much of the symptomatology of so-called G-avitaminosis or "rat-pellagra", the question arises as to whether or not some of the confusion in the vitamin G issue may not be due to the inclusion of multiple variations of which the one here described may be more or less important.

We are pursuing these studies in detail because of the appreciation that so many of the chemical and physiological properties heretofore attributed to "Vitamin G" fit in excellently with the hypothesis that the substance itself is iron. We are actively working on this problem in our laboratory from a number of angles including work with dogs, monkeys and guinea pigs.

## New York Section.

*New York Academy of Medicine, March 18, 1931.*

5461

### Spinal Cord Changes in Subacute Combined Degeneration Following Liver Therapy. (A Histopathologic Study).

CHARLES DAVISON. (Introduced by E. J. Baumann.)

*From the Neuropathology Laboratory, Montefiore Hospital, New York.*

Beneficial effects of liver therapy in pernicious anemia complicated by neurologic signs of subacute combined degeneration were reported by Minot and Murphy,<sup>1</sup> Richardson,<sup>2</sup> Ungley and Suzman,<sup>3</sup> and others. A histopathologic study of the spinal cords in

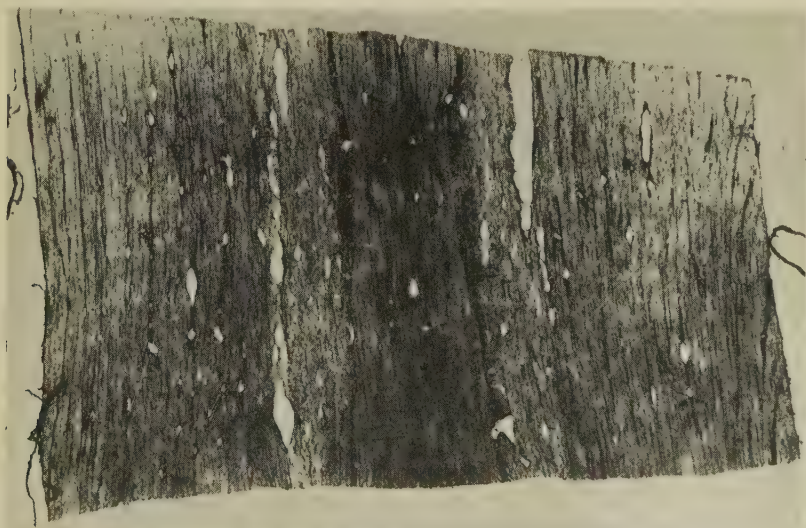


FIG. 1 (a).

Longitudinal section from a normal spinal cord through the posterior and lateral tracts showing the normal glia structure. Victoria blue stain.  $\times 30$ .

<sup>1</sup> Minot, G. R., and Murphy, W. P., *J. Am. Med. Assn.*, 1926, **87**, 470; 1927, **89**, 759.

<sup>2</sup> Richardson, W., *New Eng. J. Med.*, 1929, **200**, 540.

<sup>3</sup> Ungley, C. C., and Suzman, M. M., *Brain*, 1929, **52**, 271.



FIG. 1 (b). Same as Fig 1 (a).  $\times 480$ .

treated and improved cases of subacute combined degeneration has never been reported. The present study was limited to 7 cases of subacute combined degeneration (due to pernicious anemia) which received liver; 2 of the cases showed some improvement in the neurologic symptoms.

Transverse and longitudinal sections of the spinal cord of these cases were stained for myelin sheaths, axis cylinders and glia, and compared with sections from 10 untreated cases of subacute combined degeneration. The myelin sheaths and axis cylinders in the treated and untreated cases showed the same changes. The only histopathologic difference observed was that of the glia. In the untreated cases of subacute combined degeneration the glia destruction ran parallel with that of the myelin sheaths and axis cylinders. The poor glia response in subacute combined degeneration is designated by neurohistopathologists as "a regressive glia change". (Figs. 2a and 2b.) In the treated cases instead of a poor glia response, there was a definite increase in the glia fibers (Figs. 3a and 3b) which is designated as "progressive glia change".



FIG. 2 (a).

Longitudinal section of the spinal cord through the posterior and lateral tracts from a case of pernicious anemia, not treated with liver, complicated by neurologic signs and symptoms of subacute combined degeneration. Notice the poor glia response throughout the section and compare with Fig. 1 (a). Victoria blue stain.  $\times 30$ .

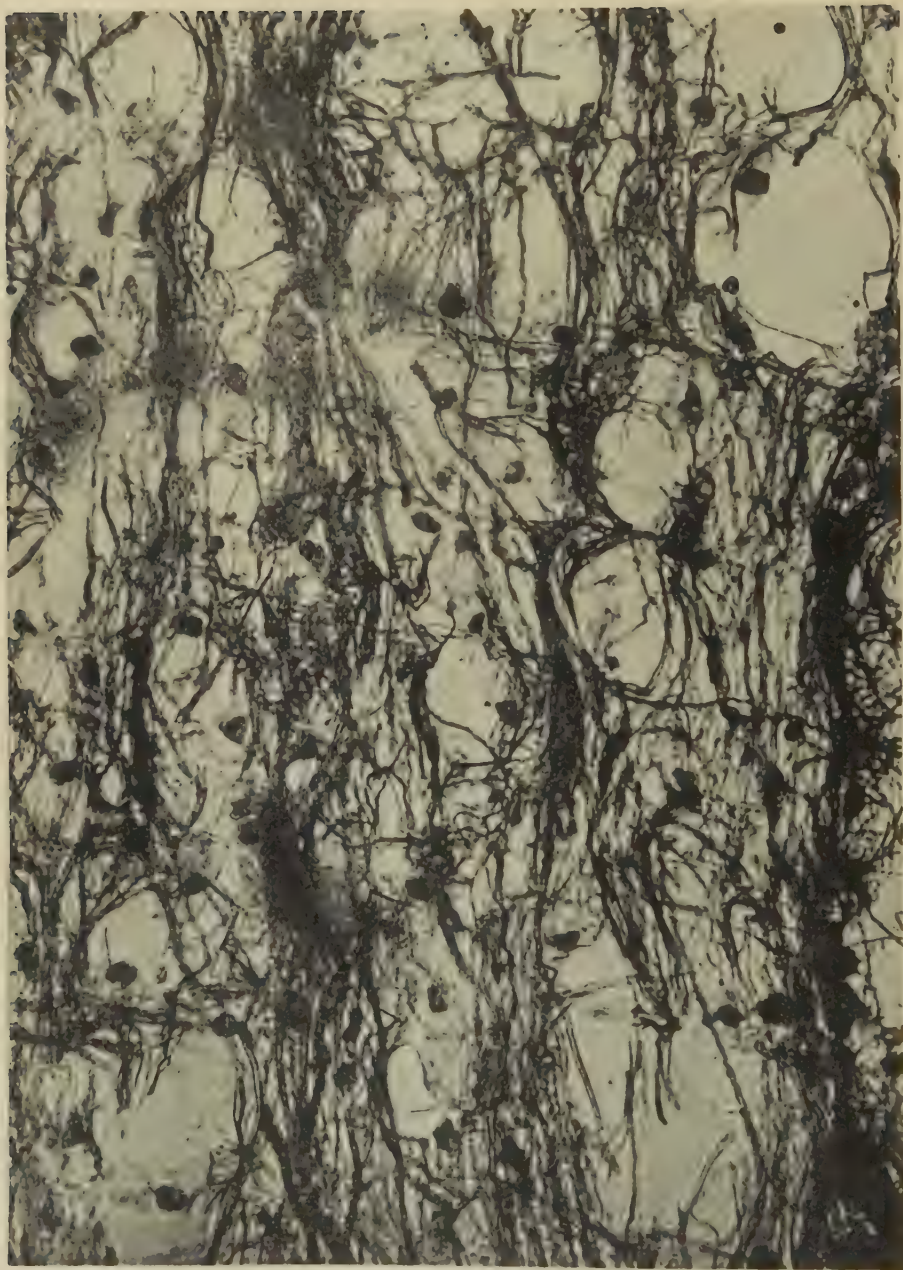


FIG. 2 (b). Same as Fig. 2 (a).  $\times 480$ .



FIG. 3 (a).

Longitudinal section of the spinal cord through the posterior and lateral tracts from a case of pernicious anemia, complicated by subacute combined degeneration, which received liver therapy. Notice the intense glia proliferation (designated by arrows) in the crossed pyramidal tracts and posterior columns and compare with Figs. 1 (a) and 2 (a). Victoria blue stain.  $\times 30$ .

From the study of these cases the intensity of the glia proliferation did not appear to depend upon the early administration nor upon the duration of treatment with liver. It may be assumed that in these cases the liver therapy either caused a reduction in the hypothetical toxin (pernicious anemia) or in its attenuation, and therefore allowed the glia to proliferate and replace the destroyed tissue. The only possible effect that liver therapy may have on the myelin sheaths and axis cylinders is to arrest the further destruction of these structures. In the light of our present day knowledge of neuro-histopathology, regeneration of destroyed axis cylinders is inconceivable. This finding, however, should not discourage us in the early administration of liver in cases of pernicious anemia with or without neurologic complications. To succeed in delaying the progress of destruction of the axis cylinders and in causing the formation of a glia scar is in itself an advantage.



FIG. 3 (b). Same as Fig. 3 (a).  $\times 480$ .

5462

# Lesions of the Nervous System Resulting from a Deficiency of the Vitamin B Complex.

HARRY M. ZIMMERMAN AND ETHEL BURACK.\*

(Introduced by George R. Cowgill.)

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Dogs subsisting on artificial food mixtures complete so far as known except with respect to the vitamin B complex have been shown by Cowgill<sup>1</sup> to develop a syndrome characteristic of lack of the antineuritic vitamin B. Histological examination of both the central and peripheral parts of the nervous system of such animals has been made. In the vagus, brachial and sciatic nerves there was revealed myelin degeneration of a patchy distribution. This demyelination could be demonstrated by the Marchi method, the Spielmeyer frozen-section method, and in Sudan stains. The severity of the pathologic process varied directly with the length of time which elapsed between the onset of the paralytic syndrome and the death of the animal. No evidence of phagocytosis or of a reparative process could be demonstrated in any of the peripheral nerves.

In 3 of the 8 animals studied focal degenerative lesions were found in the cerebrum and pons. These were characterized by destruction of ganglion cells and myelin sheaths, and an extensive proliferation of fat granule cells and blood vessels.

In one animal there was marked glial proliferation in the dorsal columns of the spinal cord similar to that observed in cases of human pellagra. The cords of all the dogs showed focal disseminated zones of demyelination like those described by Gildea, Kattwinkel, and Castle,<sup>2</sup> but inasmuch as the latter lesions were demonstrable only by the Pal-Weigert and the Spielmeyer techniques and not by the Marchi method or Sudan stains, it is possible that they are artifacts. Further experiments designed to answer this question are now in progress.

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\* Alpha Xi Delta Fellow, American Association of University Women.

<sup>1</sup> Cowgill, G. R., *Am. J. Phys.*, 1923, **66**, 164.

<sup>2</sup> Gildea, E. S., Kattwinkel, E. E., and Castle, W. B., *New Eng. J. Med.*, 1930, **202**, 523.

## 5463

### The Vitamin B Complex in Relation to Food Intake During Hyperthyroidism.

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*From the Department of Physiology, Yale University School of Medicine.*

Dogs 1 and 2 had maintained their appetite for a diet of dog biscuit for at least 3 months. After 5 gm. of desiccated thyroid were added to their daily ration they exhibited a loss of appetite and consequent loss of weight for approximately 2 weeks. During the next 3 weeks occasional administrations of vitamin B were followed by restoration of the urge to eat. The diet was then changed to the artificial one developed by Cowgill<sup>1</sup> and daily doses of vitamin B were administered, whereupon appetite was restored and the animals proceeded to regain their initial body weight.

Dogs 3 and 4 had exhibited the characteristic anorexia on the artificial food mixture in 23 and 31 days respectively. When 5 gm. of desiccated thyroid were added to their daily dietary, anorexia supervened in dog 3 in 12 days, while dog 4 lost its appetite in 21 days. These results harmonize with the theory of Plimmer,<sup>2</sup> and Cowgill and Klotz<sup>3</sup> that the amount of vitamin B required by the organism is determined chiefly by its caloric requirement.

## 5464

### Non-Toxicity of Certain Aniline Dyes for Bacteria.

JOHN W. CHURCHMAN.

*From the Laboratory of Experimental Therapeutics, Cornell Medical College.*

If 1 cc. of saturated aqueous solution of neutral red (5.6%) be put into tubes containing  $\frac{1}{2}$  cc. of heavy aqueous suspension of 24 hour culture of *Staphylococcus aureus*, *B. prodigiosus*, and *B. anthracis*, and plants be made on agar at the end of 48 hours, the organisms will grow as vigorously as if no dye had been present in the tubes. *B. prodigiosus* will be still capable of vigorous growth on

<sup>1</sup> Cowgill, G. R., *Am. J. Physiol.*, 1923, **66**, 164.

<sup>2</sup> Plimmer, R. H. A., *Brit. Med. J.*, 1926, **1**, 239.

<sup>3</sup> Cowgill, G. R., and Klotz, B. H., *Am. J. Physiol.*, 1927, **81**, 470.

transplant after 9 days' exposure to the saturated solution of the dye.

If, instead of saturated solution, 2% solution be used, all 3 organisms will grow vigorously on transplant even after 21 days' exposure, and *B. prodigiosus* and *B. anthracis* will grow well at the end of 35 days' exposure.

*Staphylococcus aureus* (now known to be highly susceptible to triphenyl methane dyes, like crystal violet) readily survives 24 hours' exposure to saturated aqueous solution of Bismarck Brown (1.2%); and *B. anthracis* (another organism susceptible to triphenyl methanes) put 39 days ago into this solution still grows vigorously when transplanted.

These observations again make clear that the statement sometimes made that a strong aqueous solution of dye stuff is *eo ipso* sterile must undergo considerable modification before it conforms with the facts.

## 5465

### Resistance of *B. Prodigiosus* to Aniline Dyes.

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*From the Laboratory of Experimental Therapeutics, Cornell Medical College.*

*B. prodigiosus* is known to be one of the organisms which are resistant to triphenyl methane dyes, as well as to other classes of dyes. The experiments here recorded indicate the—perhaps not generally realized—degree of this resistance.

Careful determinations showed that growth of *B. prodigiosus* on agar is prevented by gentian violet\* only when the dye is used in dilutions of 1 to 900. Compare this with *B. anthracis* whose growth on agar is completely inhibited by dilutions of approximately 1 to 1,000,000, and whose growth in broth is inhibited even by much weaker dilutions.

This marked resistance of *B. prodigiosus* to the *bacteriostatic* effect is paralleled by its resistance to the *bactericidal* effect of certain triphenyl methane dyes. That the organism (at least the strains of it usually encountered) readily survives exposure to gentian violet

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\* These experiments were started several years ago and at that time Grüber's gentian violet was being used under the generally held, but mistaken, notion that it was particularly "pure".

for periods of minutes or hours (as contrasted with *Staphylococcus aureus* which is killed by exposures of 45 minutes in 1 to 15,000 solution) is readily shown. Transplants made from a tube containing *B. prodigiosus* suspended in 2% gentian violet showed vigorous growth, though the organisms had been exposed to the dye for 4 hours.

The present report deals, however, with survival of *B. prodigiosus* after exposure to dye for periods of months.

In a preliminary experiment, transplants on agar made from a tube containing gentian violet (1 to 15,000) and *B. prodigiosus* showed the organism to be still alive and vigorous at the end of 14½ months. In another experiment transplants made at the end of 26½ months from a similar tube showed just as vigorous growth of the organism as if no dye whatever had been present.

With acid fuchsin, the results were even more striking. Transplants on agar from a tube containing suspension of *B. prodigiosus* set up nearly 46 months ago still show vigorous growth, though the tubes contain 4 drops of 2% aqueous acid fuchsin to 2 cc. of bacterial suspension.

These results indicate once more that the investigations of recent years have proved entirely too general, statements like those of Michaelis,<sup>1</sup> made years ago and doubtless in accord with the then known facts, that "all dye stuffs are, in high concentrations, bacteria poisons."

## 5466

### Immunological Studies in Relation to Suprarenal Gland. VII. Effect of Bilateral Suprarenalectomy on Acquired Resistance in Rats.

J. MARMORSTON-GOTTESMAN AND DAVID PERLA.

*From the Laboratory Division, Montefiore Hospital, New York.*

In previous studies we gathered evidence of the importance of the suprarenal gland in antibody formation through its influence on the water balance in the tissues of the body.<sup>1, 2, 3, 4, 5</sup> That the supra-

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<sup>1</sup> Michaelis, L., *Einführung in die Farbstoffchemie für Histologen*, 1902, 26.

<sup>2</sup> Marmorston-Gottesman, J., and Perla, David, *J. Exp. Med.*, 1928, **47**, 713.

<sup>3</sup> Perla, David, and Marmorston-Gottesman, J., *J. Exp. Med.*, 1928, **47**, 723.

<sup>4</sup> Marmorston-Gottesman, J., and Perla, David, *J. Exp. Med.*, 1928, **48**, 225.

<sup>5</sup> Perla, David, and Marmorston-Gottesman, J., *J. Exp. Med.*, 1929, **50**, 87.

<sup>5</sup> Marmorston-Gottesman, J., and Perla, David, *J. Exp. Med.*, 1929, **50**, 93.

renal glands are essential in the mechanism of *natural resistance* has been established by Marine and his coworkers in this laboratory<sup>6, 7, 8, 9, 10, 11</sup> and by other investigators.<sup>12, 13, 14, 15, 16, 17</sup> In an effort to determine the relation of the suprarenal gland to *acquired resistance* the following experiment was carried out.

In a preliminary experiment the M.L.D. of a batch of typhoid vaccine (preparation No. 120, lot No. 199) in suprarenalectomized rats was determined. This was found to be 0.5 cc.\* Fourteen rats received 3 intraperitoneal injections of typhoid vaccine at weekly intervals (0.5 cc. and 1 cc.). Five days after the last injection these 14 rats were suprarenalectomized, together with 10 normal uninjected rats. On the sixth day following the operation 8 immunized suprarenalectomized rats were injected with 5 cc. of typhoid vaccine and 6 were injected with 1 cc. intraperitoneally. All the unimmunized rats were injected with twice the M.L.D. (1 cc.).

*Results.* Within 12 hours all the unimmunized rats died. Those rats that had been immunized and subsequently suprarenalectomized survived the injection of 10 M.L.D.'s for suprarenalectomized rats. Repeated injections of typhoid vaccine prior to suprarenalectomy raise the resistance of rats to more than 10 M.L.D. for suprarenalectomized rats.

Suprarenalectomy apparently does not diminish the acquired resistance to typhoid vaccine.

It has been shown that *Bartonella muris* infection in the adult splenectomized rat cannot be transmitted to the suprarenalectomized rat of infected stock.<sup>18</sup> The rat is spontaneously infected with *Bar-*

<sup>6</sup> Jaffe, H. L., and Marine, D., *PROC. SOC. EXP. BIOL. AND MED.*, 1923, **21**, 64.

<sup>7</sup> Marine, David, *PROC. SOC. EXP. BIOL. AND MED.*, 1924, **21**, 497; *Bull. Acad. Med. Cleveland*, 1924, **7**, 1.

<sup>8</sup> Scott, W. J. M., *J. Exp. Med.*, 1923, **38**, 543.

<sup>9</sup> Flashman, D. H., *J. Infect. Dis.*, 1926, **38**, 461.

<sup>10</sup> Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

<sup>11</sup> Marmorston-Gottesman, J., Perla, David, and Vorzimer, Jefferson, *J. Exp. Med.*, 1930, **52**, 587.

<sup>12</sup> Lewis, J. T., *Rev. d. l. asoc. med. Argentina*, 1921, **35**, 529.

<sup>13</sup> Lewis, J. T., *Am. J. Physiol.*, 1923, **64**, 506.

<sup>14</sup> Belding, D., and Wyman, L. C., *Am. J. Physiol.*, 1926, **78**, 50.

<sup>15</sup> Crivellari, C. A., *Compt. rend. Soc. Biol.*, 1927, **96**, 223.

<sup>16</sup> Voegtlin, C., and Dyer, H. A., *J. Pharmacol. and Exp. Therap.*, 1925, **24**, 101.

<sup>17</sup> Steinback, M. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 142.

\* It has been shown that with different batches of typhoid vaccine the M.L.D. for suprarenalectomized rats varies. It is therefore necessary to determine the M.L.D. of each batch of typhoid vaccine used in each experiment.

<sup>18</sup> Marmorston-Gottesman, J., and Perla, David, *J. Exp. Med.*, 1930, **52**, 121.

*tonella muris* anemia early in life, between the fourth and sixth week. It then becomes a carrier of the *Bartonella muris* and possesses an acquired immunity to the infection. The infection following splenectomy in the adult carrier rat is indicative of a depression in the acquired resistance to *Bartonella muris*. It has been further shown that the acquired resistance to *Trypanosoma lewisi* of normal rats as a result of a previous infection is uninfluenced by subsequent suprarenalectomy. These observations indicate that once a cellular or humoral immunity is established to an infection or an antigenic substance this acquired resistance cannot be broken down by subsequent suprarenalectomy. Bilateral suprarenalectomy, though markedly depressing the *natural resistance* of the adult albino rat to toxins, poisons and bacterial and protozoan infections, does not affect the *acquired resistance* resulting from previous injections of such substances. Acquired and natural resistance are dependent on different physiological processes in the organism and are not merely quantitative variations of the same process as is generally assumed.

## 5467

Effect of Injections of Cortin on Resistance of Suprarenalectomized Rats to Histamine Poisoning.

DAVID PERLA AND J. MARMORSTON-GOTTESMAN.

*From the Laboratory Division, Montefiore Hospital, New York.*

In a previous communication<sup>1</sup> the protective action of injections of cortin on the resistance of suprarenalectomized rats to typhoid vaccine was reported. In these studies an extract of the cortex of the suprarenal gland, made in this laboratory according to the method of F. A. Hartman, was used. It was found that Hartman's cortin is a highly potent extract of the suprarenal cortex. It is free of toxicity and epinephrin. Repeated injections of cortin will raise the resistance of suprarenalectomized rats to several lethal doses of typhoid vaccine. It was suggested that a cortical extract may be biologically assayed by determining the minimal protecting amount to be administered within 24 hours before and after the injection of the minimal lethal dose of typhoid vaccine for suprarenalectomized adult rats on the 6th day after suprarenalectomy.

<sup>1</sup> Perla, David, and Marmorston-Gottesman, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1931, **28**, 648.

In the following experiments the effect of injections of cortin on the resistance of suprarenalectomized rats to histamine (ergamine acid phosphate, Burroughs Wellcome) was studied. Previous work<sup>2</sup> had shown that suprarenalectomized rats are highly susceptible to histamine poisoning. It was suggested that histamine could be used as a gauge of suprarenal insufficiency. Adult suprarenalectomized rats are killed by 100-120 mg. of histamine per kg. when administered on the 6th day after operation.<sup>2, 3</sup> Normal rats survive 700-900 mg. per kg.<sup>4, 5, 2</sup>

Preliminary tests in the present studies indicated that immature suprarenalectomized rats will resist larger amounts of histamine than mature suprarenalectomized rats. Six-week-old immature albino rats of Wistar stock were used. The minimal lethal dose of histamine for 6-week-old suprarenalectomized rats was determined. Twenty-four suprarenalectomized, 6-week-old albino rats were divided into 3 groups. Five received on the 6th day after operation 100 mg. per kg. of body weight of histamine, 14 received 150 mg. per kg. and 5 received 200 mg. per kg. Of those receiving 200 mg. none survived; of those receiving 150 mg., 60% survived, and of those receiving 100 mg. all survived. The minimal lethal dose of histamine for suprarenalectomized 6-week-old rats is between 150 and 200 mg. per kg. of body weight. The minimal lethal dose of histamine for suprarenalectomized adult rats is between 100 and 120 mg. per kg. of body weight. It has been found in earlier experiments that the continuous administration of epinephrin from the day of operation to the day of histamine poisoning will protect suprarenalectomized rats in about half the instances to one minimal lethal dose of histamine.<sup>6</sup> In these experiments it has been found that injections of physiological salt solution did not raise the resistance to histamine poisoning.

Injections of 0.5 cc. (1 cc. is equal to 40 gm. of cortex) of cortin were made twice daily into 14 suprarenalectomized rats. On the 6th day, 6 rats received 150 mg. of histamine per kg., 3 received 200 mg. per kg., 3 received 300 mg. per kg. and 2 received 500 mg. per kg. All of these rats survived. The daily injections of cortin raised the resistance to at least 3 minimal lethal doses of histamine. The maximal amount of histamine that suprarenalectomized rats treated with cortin will survive is being determined.

<sup>2</sup> Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

<sup>3</sup> Scott, W. J. M., *Arch. Path. and Lab. Med.*, 1927, **4**, 491.

<sup>4</sup> Crivellari, C. A., *Compt. rend. Soc. biol.*, 1927, **96**, 223.

<sup>5</sup> Voegtlin, C., and Dyer, H. A., *J. Pharmacol. and Exp. Therap.*, 1925, **24**, 101.

<sup>6</sup> Perla, David, and Marmorston-Gottesman, J., *Am. J. Phys.*, 1929, **89**, 152.

The effect of injections of cortin during the last 24-hour period before and after the injections of histamine was determined. One cc. of cortin was administered to 6 suprarenalectomized rats twice on the 5th day and on the 6th day, 2 hours prior to the injection of histamine and 2 hours after the injection of histamine. Three of these rats were given 300 mg. of histamine per kg. of body weight and three, 500 mg. per kg. of body weight. All the rats survived. The administration, therefore, of cortin during the last 24-hour period prior to the injection of histamine will raise the resistance of suprarenalectomized rats to at least 3 minimal lethal doses of histamine. (See Table I.)

TABLE I.  
Effect of Injections of Cortin on Resistance of Suprarenalectomized Rats to Histamine Poisoning at End of Second Week (6-week-old rats).

Rat No.	Cortin for 6 days after suprarenalectomy	Histamine mg. per kg. 6th day		No cortin 8-12 days after operation. Cortin given days after operation		Histamine mg. per kg. 14th day	
				13	14		
1	1 cc.	500	Survived	0	0	200	Died
2	"	500	"	"	"	200	"
3	"	300	"	"	"	200	"
4	"	300	"	"	"	200	"
5	"	300	"	"	"	200	"
6	"	200	"	2 cc.	1.5 cc.	300	Survived
7	"	200	"	"	"	300	"
8	"	200	"	"	"	300	"
9	0	150	"	"	"	300	"
10	"	150	"	"	"	500	"
11	"	150	"	"	"	500	"
12	"	150	"	"	"	500	"
13	"	150	"	"	"	500	"

An experiment was carried out to determine the protective action of cortin in suprarenalectomized rats when administered during 24 hours at the end of the second week after operation. Thirteen rats were used. Eight of these received daily injections of 1 cc. of cortin in 2 doses during the first 6 days after operation. Five rats received no cortin. Of the 8 treated rats, 2 were given 500 mg. of histamine per kg.; three, 300 mg., and three, 200 mg. per kg. on the 6th day after operation. All of these rats survived. The 5 untreated suprarenalectomized rats were given 150 mg. of histamine per kg. All the rats of this group survived. During the second week no cortin was administered to the rats of either group until the 13th day. The 5 rats that had received no cortin in the first week were given 2 cc. of cortin on the 13th day and 1.5 cc. on the

TABLE II.

Effect of Repeated Injections of Cortin on Resistance of 6-week-old Suprarenalectomized Rats to Histamine Administered on the 6th Day after Operation

No. of Rats	Cortin						Histamine mg. per kg. 6th day	No. of Rats Survived	No. of Rats Died
	Days after suprarenalectomy	1	2	3	4	5	6		
6	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	1 cc.	150	6	0
3	"	"	"	"	"	"	200	3	0
3	"	"	"	"	"	"	300	2	1
2	"	"	"	"	"	"	500	2	0
3	0	0	0	0	2 cc.	2 cc.	300	3	0
3	"	"	"	"	"	"	500	3	0
Untreated Rats									
5	0	0	0	0	0	0	100	5	0
14	"	"	"	"	"	"	150	9	5
5	"	"	"	"	"	"	200*	0	5

1 cc. extract represents 40 gm. of cortex.

\*It has been previously demonstrated that adult suprarenalectomized rats are killed by 100-120 mg. of histamine per kg. on the 6th or 7th day.<sup>2, 3</sup>

14th day. Three rats which had received daily injections of cortin in the first week were given 2 cc. on the 13th day and 1.5 cc. on the 14th day. On the 14th day, 4 of these rats received 300 mg. of histamine per kg. and 4 received 500 mg. per kg. All 8 rats survived. Five rats that had received cortin during the first week received no cortin in the second week. On the 14th day these rats were injected with 200 mg. of histamine per kg. of body weight. All were killed within 3 hours.

These experiments further demonstrate that repeated injections of cortin raise the natural resistance of suprarenalectomized rats to poisons as well as bacteria. It is evident that the marked susceptibility of suprarenalectomized rats to histamine poisoning is not due to epinephrine insufficiency as maintained by Wyman but to cortical insufficiency.

It is suggested that the protective action of cortical extracts against histamine poisoning in suprarenalectomized rats is a better means of biologically assaying the potency of such extracts than typhoid vaccine which we suggested in a previous communication. Ergamine acid phosphate (histamine) is a standard chemical that varies very little in toxicity. The amount of cortical extract injected intraperitoneally into suprarenalectomized albino rats on the 5th and 6th day after operation necessary to protect these rats against 200 mg. of ergamine acid phosphate (Burroughs Wellcome) may be considered as a standard unit.

## The Cellular Reaction in Experimental Syphilis. Supravital and Fixed Material.

L. PEARCE AND P. D. ROSAHN.

*From the Laboratories of The Rockefeller Institute for Medical Research.*

The blood and tissues of rabbits inoculated with *Treponema pallidum* have been studied from the standpoint of the cellular reaction at different periods of the disease. The evidence from successive weekly blood counts on 5 groups of rabbits, 40 animals in all, indicated that the various classes of cells were numerically affected. A striking finding was the increase in the numbers of circulating monocytes<sup>1</sup> associated with actively developing lesions. The increase was very much more pronounced than the spontaneous rises of monocytes noted in groups of normal rabbits, the blood of which was examined at similar intervals over prolonged periods of time.<sup>2</sup>

Similar experiments have been carried out in which various tissues involved by the disease process were examined, both in supravital and in fixed preparations, in order to study the relationship between the blood picture and the types of cells concerned in the tissue reaction. Supplementary observations were also made on a large number of syphilitic rabbits in which the blood was not examined systematically; every instance of tissue examination, however, was immediately preceded by a blood count. Standardized pipettes were used and the differential counts were made with the supravital technique. Scrapings of fresh tissue were examined by the supravital method, using neutral red and Janus green dyes, and fixed preparations of the tissues were made with various fixatives and stains. In all experiments, special attention was paid to the clinical character of the disease. The particular lesions studied and the time selected for their examination was largely determined by the general course of the infection, and it should be emphasized that an essential feature of the study was the examination of lesions at different stages of development. The lesions studied included the primary orchitis and periorchitis resulting from testicular inoculation as well as the metastatic orchitis of the uninoculated testicle, generalized lesions of the periosteum, cutaneous lesions of metas-

<sup>1</sup> Pearce, L., *Trans. of the VIII International Congress on Dermatology and Syphilology*, 1930, in press.

<sup>2</sup> Pearce, L., and Casey, A. E., *J. Exp. Med.*, 1930, **51**, 83; **52**, 23, 39, 145, 167.

tatic origin and those resulting from scrotal implants, and inguinal and popliteal lymph nodes.

The results at present show that during the phase of an early orchitis or of an early scrotal chancre when a peripheral monocytosis is beginning to appear, the most conspicuous cell observed in the fresh tissue preparations of these lesions is one that cannot be distinguished from a blood monocyte on the usual morphological grounds. A few clasmatoocytes are seen but they are far less numerous. With more advanced testicular and chancre lesions, the clasmatoocytes become increased and in addition, the number of lymphocytes is increased. Regressing lesions are characterized by a large proportion of clasmatoocytes and lymphocytes. In spite of the difficulties of examining fresh preparations of early generalized lesions because of their small size, the observations which have been made are in keeping with those of an early orchitis and chancre. The findings with respect to well developed generalized lesions of the periosteum and skin in various parts of the body correspond to those of the testicle and scrotum. A study of fixed material has confirmed the results obtained with preparations of fresh tissue with respect to the large mononuclear cells in early lesions. It should be stated, however, that the numbers of lymphocytes and plasma cells appear to be greater in fixed than in supravital preparations. As far as the inguinal and popliteal lymph nodes are concerned, comparatively large numbers of monocytes were found during actively progressing phases of lesions in their drainage areas.

In supravital preparations of tissues of early lesions, particularly of an orchitis, the large mononuclear cells have a variable appearance. The majority appear to be typical blood monocytes of the proper size with the characteristic indented nucleus and a rosette of neutral red, but there are also many cells with various modifications leading to a type which is ordinarily classified as a clasmatoocyte. In cutaneous lesions, the number of eosinophiles seen in supravital preparations is quite striking.

It seems highly probable that the large numbers of monocytes present in the tissues of syphilitic lesions furnish the source for the peripheral blood monocytosis, and from this standpoint, the monocyte appears as an important and perhaps one of the most essential participants in the cellular reaction to *Tr. pallidum*. The participation of the clasmatoocyte, on the other hand, particularly in association with older lesions, may be referable to secondary factors, such as degenerative changes or necrosis. In Morgan's<sup>3</sup> opinion, the clas-

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<sup>3</sup> Morgan, H. J., *Trans. Am. Phys.*, 1930, **45**, 69.

matocyte is the important cell in the cellular pathology of testicular and scrotal lesions of acute experimental syphilis.

The observations on other aspects of the cellular reaction in experimental syphilis will be presented in future communications.

## 5469

## Amytal on Smooth Muscle.

CHAPMAN REYNOLDS.

*From the Department of Pharmacology, Marquette University School of Medicine, Milwaukee, Wisconsin.*

While using sodium amytal (sodium iso-amyl-ethyl barbiturate) as a routine laboratory anesthetic, it was observed that some of the dogs seemed to show certain signs of increased intestinal motility. Gruber<sup>1</sup> found the tone of isolated intestine, uterus and ureter diminished by the barbituric acid derivatives (uterus by 1:10,000 or stronger amytal solution). Drabkin *et al.*<sup>2</sup> reported maintenance of the rhythmic contractions and of response to the oxytocic principle of pituitary by the isolated uterus following large doses of amytal (1:1,000).<sup>3</sup> Swanson<sup>4</sup> obtained only a depression of the tone of all smooth muscle structures (by 1:25,000 sodium amytal or stronger).

As these observations are somewhat in conflict, a series of experiments was done with smooth muscle tissues from various animals. The animals were killed by exsanguination and the desired parts removed at once and placed in iced Tyrode's or Locke's solution, where they were kept 2-4 hours. (In one or two instances where ether had been used a longer cold waiting period was found to improve the behavior.) The physiological bath chamber contained 200 cc. Precautions were observed as to constancy of temperature, oxygenation and pH.

The first experiments showed only depression by sodium amytal. On using smaller and smaller doses of the drug, however, a consistent "reversal effect" was obtained with every specimen that offered a uniform and dependable activity. Concentrations of 1:100,000-1:40,000 caused a prompt and vigorous increase in tone,

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<sup>1</sup> Gruber, Charles M., *J. Pharm. and Exp. Ther.*, 1927, **30**, 149.

<sup>2</sup> Drabkin, D. L., Ravdin, I. S., and Hirst, J. C., *Am. J. Med. Sci.*, 1929, **178**, 379.

<sup>3</sup>, <sup>4</sup> Personal communications.

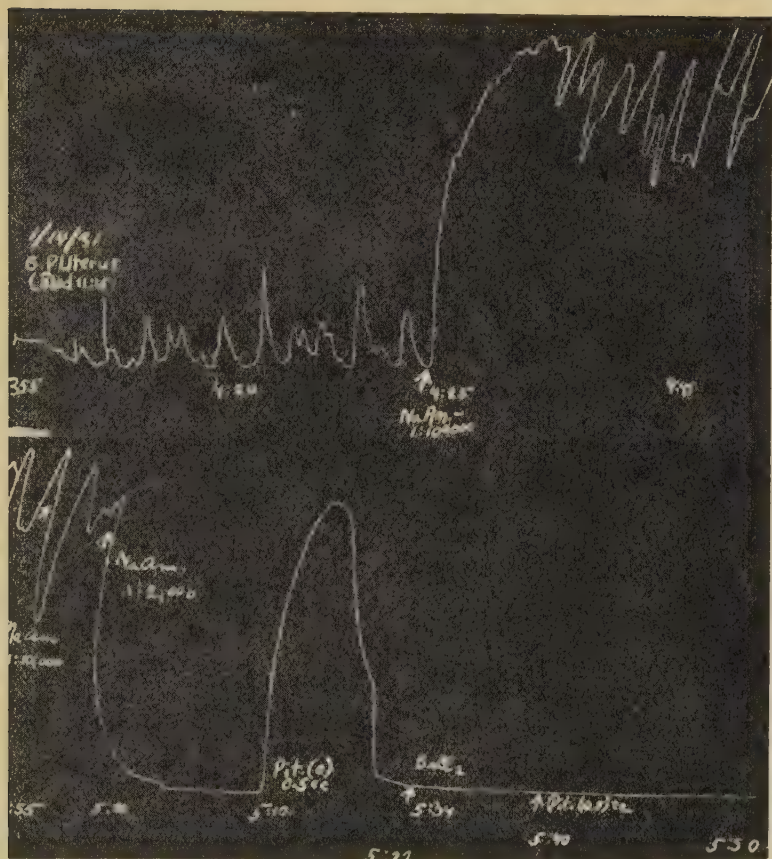


FIG. 1. (Lower tracing is a continuation of the upper.)

The effect of various concentrations of sodium amytal on one horn of the virgin guinea pig uterus in oxygenated Locke's solution. The uterus had previously been in iced solution 4 hours and 40 minutes. Sodium amytal, 1:100,000, induced an increased tonus that was maintained for 30 minutes; this was slightly alleviated by 1:10,000, and abolished by 1:2,000, as was all rhythmic activity. After this treatment, 0.5 cc. pituitrin O caused one contraction, immediately followed by relaxation to a base line which was not altered by a massive dose of barium chloride or by another dose of pituitrin of the same size.

too often sending the writing point above the paper, although the lever magnification was only 3:1 and the segment a short one. This high tone was generally accompanied by an increase in the height of individual contractions. This rise was maintained for at least 30 minutes unless a change was made in the content of the bath. A large dose of sodium amytal, introduced during this period, was followed by an immediate relaxation to control level or lower and, if large enough, an abolition of individual contractions. Figure 1 is a typical tracing. Primary concentrations of 1:25,000-1:1,000

markedly lowered the tone level and, with the stronger ones, completely abolished rhythmic activity. A high tone previously induced by pituitrin was immediately brought back to control level or lower by these concentrations. Following a primary dose of sodium amytal in this range the tissue responded to pituitrin either in a curtailed fashion or not at all, and usually was unable to respond even to barium.

While the quantitative reaction varied considerably, and those of the uterus were the most spectacular, the directional responses were consistent. Specimens used included guinea pig uterus (virgin and pregnant), rabbit duodenum, rabbit uterus (non-pregnant), kangaroo\* uterus and intestine, and portions of the digestive tract of the barn owl.

## 5470

### Delayed Differential Counting of the White Blood Cells by a Modified Supravital Technique.

ALBERT E. CASEY AND PAUL D. ROSAHN. (Introduced by L. Pearcee.)

*From the Laboratories of The Rockefeller Institute for Medical Research.*

It is a common experience in doing differential white blood cell counts by the supravital method<sup>1, 2</sup> that the cells cannot be identified after about 2 hours in the hot-box, since by this time nearly all the cells have taken up large amounts of the dye.

It has been found that by a relatively slight modification of the usual technique, differential counts may be made with great facility as long as 12 to 24 hours after taking the blood. This has been accomplished (1) by reducing the concentration of the dye used, (2) by placing the blood smears in the refrigerator until ready for counting, and (3) by eliminating the hot-box. Through the use of this procedure, all cells have been found to be actively motile and to retain their morphological viable characteristics; a 24 hour preparation which has been kept in the icebox appears to be entirely similar to a fresh smear and cannot be distinguished from it.

Thirty drops of a saturated solution of neutral red iodide No. 2

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\* This was an old female which had been in paralytic shock for 3 days. She and some of the other animals were available by courtesy of the Milwaukee Zoo.

<sup>1</sup> Simpson, M. E., *Univ. of Calif. Pub. in Anat.*, 1921, **1**, 1.

<sup>2</sup> Sabin, F. R., *Johns Hopkins Hosp. Bull.*, 1923, **34**, 277.

in 10 cc. of absolute alcohol is a satisfactory concentration for rabbit's blood instead of the usual 50 to 100 drops; as much as 100 drops per 10 cc. of absolute alcohol has been used with success in the case of normal human blood. Smears left in the open laboratory were found to last about four hours or twice as long as those kept at 37° in the hot-box. Further reduction of the temperature to 5°-10° (refrigerator) effected the preservation of the cells for as long as 24 hours. When the preserved smears are examined with an ordinary electric bulb as the source of illumination, active motility of the white cells is evident. Consequently, it has been found possible to dispense with the hot-box.

Parallel observations on 6 male rabbits were made. A series of 16 smears were counted immediately after taking the blood, with the usual technique, and a duplicate set of smears was counted after 24 hours in the icebox with the modifications here reported. In making this comparison, more than 5,000 white cells were counted upon the total of 32 smears. The means and standard errors of the means of the two series of counts are given in the following tabulation:

TABLE I.

Age of Smear in hours	Neutrophiles		Basophiles		Eosinophiles		Lymphocytes		Monocytes	
	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24	0-2	18-24
Means	59.0	57.6	3.6	4.7	0.9	0.8	23.4	24.7	12.5	12.0
Standard error of the mean	±2.1	±1.4	±0.4	±0.6	±0.2	±0.2	±2.2	±1.9	±1.1	±1.4

That comparable results were obtained is seen from the fact that no significant difference between the respective means was obtained, and in no case was this difference equal to twice its standard error. A number of counts upon normal human blood has also been made with similar results.

The time limits to which blood smears may be successfully preserved have not yet been worked out, and dyes other than neutral red have not been used; but it seems that the present studies justify the statement that accurate differential white cell counts may be made with the supravital technique as long as 24 hours after making the preparations.

### Effect of Sulphydryl Compounds and dl-Alanine on Rate of Development of Eggs of Physa and Lymnaea.

ROBERT GAUNT.\* (Introduced by E. G. Conklin.)

*From the Biological Laboratories, Princeton University.*

Hammett<sup>1, 2, 3</sup> has recently proposed that the sulphydryl ( $-SH$ ) group is "an essential stimulus to cell proliferation." He developed the idea from experiments in which an increased growth-rate by means of accelerated cell-division occurred in *Zea mays* root tips and in *Paramecium* as a result of treatment with sulphydryl compounds. In later work, Hammett and Reimann<sup>4, 5</sup> found similar compounds stimulating cell proliferation in the wounds of rats and man.

By making gross length experiments, mitotic counts and cell measurements, Hammett found that treated *Zea* root tips grew more rapidly than those of control plants and that this was the result of more rapid cell division rather than an increment in cell size. In *Paramecium* he also reported a markedly increased division rate in the treated organisms. His controls were grown in culture solutions prepared with equivalent concentrations of some related substance lacking the  $-SH$  group. For instance, his experiments on thio-glycollic acid (with some exceptions) were controlled with glycollic acid, and those with cysteine were controlled with alanine. In this way he sought to demonstrate the specificity of  $-SH$  as the stimulating factor. His results were very consistent and in every case where the reduced  $-SH$  grouping was present in a certain optimum range of concentration he found marked acceleration of division. Thio-glycollic acid, Na-dithio-diglycollate, cysteine, glutathione and cystin (to a slight extent) were reported as effective compounds in stimulating cell division. On the basis of these experiments Hammett stated the hypothesis that the  $-SH$  group is universally a "cell division hormone."<sup>1</sup>

Inasmuch as this hypothesis, if true, is one of profound biological

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\* This problem was suggested by Professor E. G. Conklin; and for his generous advice and assistance the author is grateful.

<sup>1</sup> Hammett, F. S., *Protoplasma*, 1929, **7**, 297.

<sup>2</sup> Hammett, F. S., *Proc. Am. Phil. Soc.*, 1929, **68**, 151.

<sup>3</sup> Hammett, F. S., *Arch. Path.*, 1929, **8**, 575.

<sup>4</sup> Hammett, F. S., and Reimann, S. P., *J. Exp. Med.*, 1929, **50**, 445.

<sup>5</sup> Reimann, S. P., and Hammett, F. S., *PROC. SOC. EXP. BIOL. AND MED.*, 1929, **27**, 20.

import, I set out to test the effect of some of these compounds on the development of the fresh water snails, *Physa heterostropha* Say, and *Lymnaea columella* Say. This is desirable material on which to work because the eggs can be obtained immediately after laying in the one-celled state and normally the rate of development of all the eggs of any one laying is remarkably constant. The egg masses can be cut into 2 or 3 pieces and each piece given a different treatment, with the assurance that under similar conditions they would all hatch at very nearly the same time.

Two criteria as to the relative rate of development of the controls and the treated eggs were tried. The first few cleavages may be followed by actual count, and with a small series this index was used. But since it was only a matter of a few hours until there were too many cells to count, this method was abandoned as inadequate. The time of hatching of treated and of control embryos was used as a criterion. This permitted treatment for 6 or 7 days, under laboratory conditions. An embryo was described as hatching when it had broken through the egg membrane. This entails microscopic examination, because after hatching an embryo may remain for some time on the egg mass and appear unhatched to the naked eye. To divide an egg mass into exactly 2 equal quantities was very difficult, consequently they were approximately halved and the average hatching time in any one solution was recorded as the hatching time of the mass.

The principal sulphhydryl compound used was cysteine, procured fresh from the Eastman Kodak Company laboratories at frequent intervals. This product comes as cysteine hydrochloride. Concentrations ranging from  $10^{-3}$  to  $5 \times 10^{-9}$  g. sulphur per cc. as cysteine were tried in about 200 experiments.

A mass of eggs in a 1-, 2- or 4-celled stage would be cut in two and one part placed in filtered stream-water and the other part dropped into a known concentration of the -SH compound. The -SH solutions were made up in the filtered stream-water and both control and test solutions identically buffered with phosphate buffers to a pH of 6.8. Fresh solutions were made daily and the eggs changed to 15 cc. of the freshly-prepared solution each day. This guarded against the instability of the compounds and prevented any accumulation of waste products. Care was taken that in the preparation of the solutions they should not become alkaline because of the well-known instability of the -SH group in alkaline solutions. The cultures were kept in a closed cupboard in an evenly heated

room so that any difference in temperature between control and test cultures was negligible.

It was found that a concentration of cysteine of  $5 \times 10^{-5}$  g. S. per cc. was slightly but definitely toxic. The more concentrated the solution the greater the toxicity noted. According to Hammett, the stimulating range of concentration is to be found in a dilution just below the toxic concentration. We were unable, however, to find any concentration in which the eggs would hatch faster than in the control cultures. In solutions diluted below the toxic level, control and test eggs developed almost simultaneously and any difference in hatching time was easily within the range of individual variation. This was in marked contrast to the precision of results noted when a slightly toxic culture solution was used, where although the treated eggs might lag in hatching by only a few hours an almost invariable difference was found.

In view of these negative findings Dr. Hammett was consulted and he suggested that reliable results could not be obtained unless the control solutions were made up with an equivalent concentration of a related compound lacking  $-\text{SH}$ . Consequently dl-alanine was obtained from Eastman Kodak Company and a series of experiments run, in which the alanine, made up in stream-water, was used as controls. This, too, was buffered to a pH of 6.8 as the previous controls had been.

In this series the cysteine-treated eggs hatched faster than the alanine-treated controls when a concentration of  $4 \times 10^{-5}$  g. S. per cc. or stronger was used. In other words, if the controls were adequate there was an actual stimulation of development by treatment with cysteine. But it was also noted that this apparent stimulation was marked in the series where cysteine to a concentration of  $5 \times 10^{-5}$  g. S. per cc. was used. And previous experiments had shown that such a concentration was slightly toxic when compared to stream-water controls. Therefore, it seemed probable that both substances were toxic, but one slightly more so than the other.

That such was the case was easy to demonstrate in another series of experiments in which egg masses were divided into 3 parts and one part cultured in cysteine solution, another in alanine solution, and the third in buffered stream water. With a concentration of  $5 \times 10^{-5}$  g. S. per cc., the stream-water cultures hatched first, the cysteine cultures second and the alanine-treated ones last. In 29 experiments with this concentration the stream-water cultures hatched first in 24 cases; and in 20 cases the cysteine cultures hatched

before the alanine-treated eggs. A more dilute concentration of  $2 \times 10^{-5}$  g. S. per cc. was below the toxic range of both compounds.

Thus it appears that for these forms the use of alanine controls would lead to entirely erroneous conclusions. Hammett states that all of his *Paramecium* experiments were controlled by this method, i. e., control solutions were made up of some compound lacking -SH that was as nearly as possible related to the compound being tested. In all cases in which cysteine was used in his experiments on root tips he states that alanine controls only were used. However, in his experiments on root tips with thio-glycollic acid, some were controlled with glycollic acid and others were not. He got a stimulation in either case.

In an earlier series of experiments done in the spring of 1930 the effect of thio-glycollic acid on the development of the eggs of *Physa* and *Lymnaea* was studied in the same manner as the cysteine experiments described above, except that only stream-water controls were used. With this compound likewise no concentration was found that would stimulate the rate of development above that of the controls.

These results are in conformity with those of Dr. Sun<sup>6</sup> who was unable to accelerate the rate of cell division in the sea-urchin egg by treatment with  $H_2S$ .

In a private communication Dr. Hammett raised the objection that the presence of a jelly mass around the eggs might in some way prevent the entrance of -SH as such. He suggested that perhaps there were enzymes present which oxidized the cysteine before it got to the egg. This is a possibility, but it seems improbable. It can be demonstrated that at least part of the cysteine molecule goes almost immediately into the egg. When an unbuffered solution of fairly high concentration is used, it penetrates the egg and coagulates the albuminous part within 2 or 3 minutes after they are dropped into it. That the -SH is oxidized before this entry is made seems improbable.

These results, as well as the experiments of Sun, indicate that the sulphhydryl group is not invariably a stimulus to cell division. They also call into question the legitimacy of the use of controls cultured in solutions of compounds even closely related to the compound being tested.

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<sup>6</sup> Sun, T. P., *Anat. Rec.*, 1930, **47**, 309 (Abstract).

5472

**Changes in Body Weight in Pregnancy.**

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HELEN MATTHEWS.

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Some 10 years ago, the senior author began a study of certain phases of the metabolism in normal pregnancy as a basis for a later comprehensive investigation of the toxæmias incident to this condition. Many of the measurements could demonstrate their real significance only if presented as relative values or changes, using convenient and easily ascertained biometric magnitudes as bases of reference. A case in point is the so-called basal metabolic rate, all of the usual prediction criteria involving some expression of the body weight in the formula used. A review of the literature disclosed a paucity of dependable recent observations of weight changes, and the few older records were incomplete. Single measurements on isolated cases are of little worth. Only continuous studies, both ante- and post-partum, on the same individual, are significant. This involved the continued cooperation on the part of the individual patient. In the present study the work has been carried out on this basis.

For the purposes of the entire investigation, subjects were drawn from 2 independent sources, group "A" from a prenatal out-patient service, supplemented by a few private cases, groups "B" and "C" from inmates of 2 nursing homes for unmarried mothers. The living conditions of the 2 major divisions thus represent the extremes of possible practice.

Normal, healthy individuals were selected initially and only those retained who maintained this condition throughout the period of study. Considerably more than 200 individuals were at least partially studied to yield the 77 that form the basis of this report.

The present paper will be devoted to the record of weight changes. Weights were taken at a uniform hour in the early morning, after emptying the bladder, with the patient nude and in a post-absorptive state (i. e. at least 12 hours after the last meal). No allowance could be made for variations in the amount of the intestinal contents. A standard precise, platform type of scale was used, the accuracy of which was checked at intervals. All measurements were made in duplicate and checked independently.

With a series of consecutive measurements on each individual patient, the weight changes are susceptible to a somewhat detailed analysis. See Table I.

TABLE I. *Weight Relations.*

Datum	Group			Average or Total
	A	B	C	
Number of patients	<b>25</b>	<b>21</b>	<b>31</b>	<b>77</b>
Av. Period of Observation, A.P. (weeks)	<b>21</b>	<b>8</b>	<b>14</b>	<b>15</b>
Gain per week				
High (kg.)	0.74	1.03	0.90	1.03
Low "	0.15	0.02	0.15	0.02
Average "	<b>0.32</b>	<b>0.52</b>	<b>0.54</b>	<b>0.46</b>
Weight, 1 week A. P.				
High (kg.)	84.8	81.2	87.0	87.0
Low "	53.4	48.4	49.8	48.4
Average "	<b>67.0</b>	<b>64.4</b>	<b>66.8</b>	<b>66.3</b>
Weight, P. P.				
High (kg.)	76.0	74.6	74.9	
Low "	43.1	40.4	43.2	
Average "	<b>57.8</b>	<b>57.1</b>	<b>57.2</b>	<b>57.4</b>
Average interval (wks)	<b>2</b>	<b>3.5</b>	<b>5</b>	<b>3.5</b>
Total loss in weight (kg.)	<b>9.2</b>	<b>7.3</b>	<b>9.6</b>	<b>8.9</b>
Weight of child.*				
High (kg.)	4.32	4.09	4.04	4.32
Low "	2.41	2.00	2.38	2.00
Average "	<b>3.31</b>	<b>3.20</b>	<b>3.28</b>	<b>3.27</b>

\* This is net weight of child alone. Placenta and other factors to be considered later.

Group "A" (from the Prenatal Clinic) shows a definitely smaller average gain per week than do the 2 institution series. That this cannot be ascribed entirely to more rapid weight increment in the later stages of the pregnancy is shown by the fact that Group "C," although averaging a period of study nearly twice that of the comparable Group "B," still has a slightly larger increase. Group "A" contained many multiparae; they were living at home, were responsible for the cares of households, and from the social status of the majority, probably under a somewhat less liberal dietary regimen than were the institutional group. Further, the latter, while they performed certain domestic duties, were certainly not as actively employed as were the housewives of Group "A." The point is stressed as it is one of the few real differences between the 2 major groups which this study has disclosed. With the limited data, 0.3 kilo to 0.6 kilo, may be offered as a first approximation of the average weekly weight change under varied conditions at least during the last 5 months of pregnancy. Individual fluctuations in this presumptively normal group fall well outside of these boundaries.

The level of protein utilization of the patient is indicated quantitatively by the total nitrogen output in a 24-hour collection of urine. Further, experience has shown that with adequate protein

ingestion, the remainder of the diet can safely be assumed to meet the needs of the individual. The converse does not hold since the subject with partial protein starvation may utilize a sufficient excess of fat and carbohydrate to produce and maintain a marked obesity. It has long been recognized that the pregnant woman stores protein, significantly in excess of the foetal requirement. In the present study, the average weekly total nitrogen output ante-partum ranged from 6.73 gm. to 9.64 gm. for 24 hours with a total average for the period of 8.03 gm.<sup>1</sup> While this is but slightly above the maintenance level, it must be remembered that the women were anabolizing a further amount of nitrogenous food. It seems warrantable to conclude that these patients were adequately nourished. Possible additional support is derived from the average level of 8.04 gm. of nitrogen eliminated post-partum; the agreement of the 2 total average values being, of course, fortuitous. Post-partum an appreciable amount of metabolized nitrogen is being eliminated as protein in the breast milk and again there is no evidence of any nutritional inadequacy.

The body weight of the individual patients showed a fairly steady upward trend during the period of study. This can best be illus-

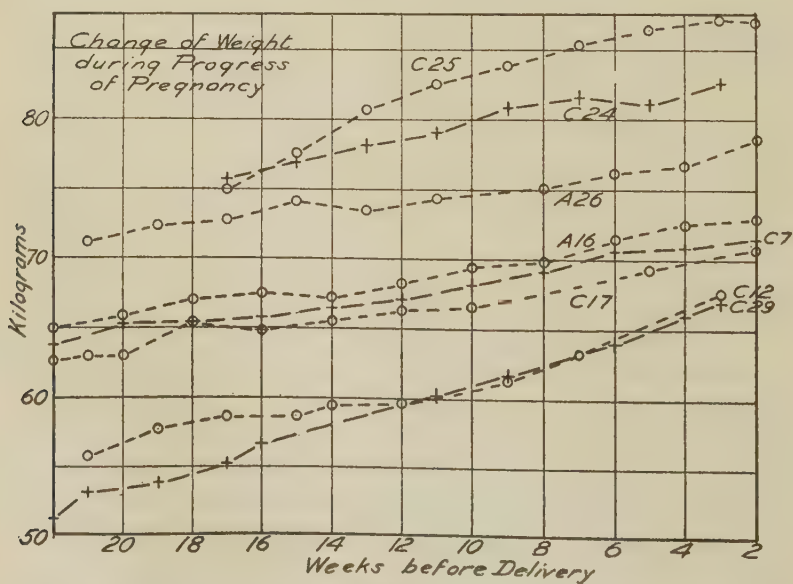


FIG. 1.

Periodic changes in weight of several individuals during the course of pregnancy.

<sup>1</sup> Rowe, A. W., Gallivan, D. E., and Matthews, H., *Am. J. Physiol.*, 1930, **95**, 592.

trated graphically from the data of a few characteristic protocols. (See Figure 1.)

The average weights of the sub-groups, both ante- and post-partum, show excellent correlation. The figures after delivery are not strictly comparable, as already noted, as the time of initial access to the patients after confinement varied materially as the result of attendant conditions. Group "A" were examined before discharge from the hospital after delivery, and in some cases as they returned at later intervals to the Post-natal Clinic. Group "B" were in an institution which combined the home and the maternity hospital in one plant with separate buildings, while the patients in Group "C" were transferred from the home to a sister institution for delivery and first became available for continuance of the study after their return to the home several weeks later.

The total weight losses were somewhat variable, particularly as the average net child weights were practically identical in the 3 sub-groups. This latter fact is interesting in view of the divergent values for the maternal weight increment already considered above. It is planned to make a more detailed study of the weight losses during the actual delivery and their allocation to the several factors involved. The present data warrant only the conclusion that of the very considerable weight loss, that of the child may constitute from 30% to 45% of the whole.

*Conclusion.*—1. Groups of pregnant women, maintained under divergent domiciliary conditions, have been studied continuously during both the ante- and post-partum periods. 2. Increments in weight averaged 0.46 kilo per week for the last 15 weeks. The individual variations were large. 3. Decrements post-partum showed an average value of nearly 9 kilos of which the average weight of the child was about one-third. During this period, also, the individual changes showed a wide scatter from the mode.

The authors take much pleasure in acknowledging their indebtedness to the staffs of the several institutions and to the patients who cooperated so helpfully in the study.

**Studies on a Urinary Proteose. I. Skin Reactions and Therapeutic Response to Injections.**

WARD DARLEY AND RICHARD WHITEHEAD.

(Introduced by Robert C. Lewis.)

*From the Departments of Medicine, Physiology and Pharmacology, University of Colorado School of Medicine, Denver, Colorado.*

Oriel and Barber<sup>1</sup> recently reported the recovery of a proteose from the urine excreted in anaphylactic and allergic conditions which gave positive intradermal reactions and which gave encouraging results when used for desensitization.

We have studied the proteose reactions in 34 cases from the medical wards of the Colorado General Hospital. The proteose was prepared according to the method of Oriel and Barber. We have found that no constant relationship can be shown to exist between the quantity of proteose obtained from the urine and the responsiveness to it on the part of the patient. A large quantity may give a negative reaction when tested intradermally; on the other hand, a small precipitate may give a marked reaction when similarly tested.

Nine cases of bronchial asthma were studied and these gave the most satisfactory results. Three of the patients were studied when asymptomatic and the intradermal tests were negative. Later 2 of these patients returned to the hospital with acute asthmatic paroxysms. Proteose was again prepared and positive skin tests were obtained. Two out of 4 cases reacted negatively to the usual routine sensitization tests, but reacted positively to their respective proteose preparations. Subcutaneous injections induced focal reactions in 4 instances. Desensitization with the proteose apparently resulted in improvement in 6 out of the 9 cases of asthma studied.

Because of the recent interest in arthritis from the allergic standpoint, the proteose sensitivity was investigated in various arthritic and allied conditions. Five patients with acute arthritis were studied. In 3 instances positive intradermal tests were obtained, but the therapeutic results were questionable. A positive test was obtained in one patient with acute subdeltoid bursitis. The initial subcutaneous therapeutic injection produced a marked focal reaction which was followed by improvement. Negative intradermal tests and therapeutic results were obtained in one case of

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<sup>1</sup> Oriel and Barber, *Lancet*, 1930, p. 231.

gout, one of acute infectious arthritis, one of acute rheumatic fever, and ten of chronic arthritis.

Positive intradermal reactions were observed in 2 out of 3 cases of serum sickness. Negative results were obtained in one case of chronic generalized eczema, one of subsiding urticaria of unknown etiology, and one of erythema multiforme.

Preliminary work indicates that following the subcutaneous injection of this proteose there is a marked increase in the leucocytes of the blood with a corresponding increase in the percentage of eosinophiles.

Further work upon the therapeutic efficacy of desensitization with this urinary proteose and the blood picture following its subcutaneous injection is in progress.

#### 5474

### The Movement of Substances in the Cerebrospinal Fluid.

DONALD J. SIMONS AND MAURICE J. DIONNE.

(Introduced by Harvey Cushing.)

*From the Laboratory of Surgical Research, Harvard Medical School.*

The authors undertook to determine and collate the glucose tolerance curves in 2 loci in the cerebrospinal system and in the blood, in the hope not only of establishing the relations of the absolute sugar levels but also of obtaining data on the movement of the cerebrospinal fluid itself or of substances dissolved therein.

To determine the carbohydrate tolerance curves a healthy adult dog was used for each experiment. After 18 hours' fasting, anesthesia was induced by intraperitoneal injection of sodium amytal (Lilly). With full surgical asepsis both cerebral hemispheres were exposed by a midline trephine opening so that either lateral ventricle could be tapped with a specially made brain needle. A cisternal needle was then introduced into the *cisterna magna* and left there throughout the experiment. Since the latest modification of Folin's micro-sugar method,<sup>1</sup> using unlaked blood, was used, it was necessary to obtain from each locus only 0.1 cc. of fluid for each determination. After obtaining simultaneously one sample from each point, 20 gm. of glucose were given by vein. Every 15 minutes

<sup>1</sup> Folin-Svedberg, *J. Biol. Chem.*, 1930, **88**, 77.

thereafter for a period of  $2\frac{1}{2}$  hours, samples were taken simultaneously from each locus.

Twenty-two experiments were performed. The following table summarizes the relevant essentials from experiments performed after the technique had been perfected.

TABLE I. *Summary of Relevant Findings.*

Dog No.	Blood			Ventricle			Cistern		
	Initial value	Peak value	Min. after glucose	Initial value	Peak value	Min. after glucose	Initial value	Peak value	Min. after glucose
17	117	358	5	103	198	15	102	187	54
18	89	400	5	—	—	—	—	—	—
19	104	323	5	102	156	15	79	152	68
20	123	386	5	120	200	15	117	208	37
21	129	312	5	—	—	—	—	—	—
22	80	267	5	80	—	—	80	140	45
Average	107	344	5	101	185	15	100	172	49
Mean deviation	$\pm 18$	$\pm 50$	5	$\pm 16$	$\pm 24$	15	$\pm 19$	$\pm 29$	$\pm 14$

The data obtained appear to the authors to warrant the following tentative conclusions. These are derived for the most part from the average figures shown in Table I.

1. The relatively close approximation of the 3 initial sugar levels suggests that under stable fasting conditions the sugar level of the dog's cerebrospinal fluid equals that of the blood.

2. From the close time sequence of the maxima of the blood and ventricular sugar curves, it is evident that the ventricular fluid is affected by changes in the blood sugar level.

3. Comparison of the time relations of the maxima of the ventricular and cisternal sugar curves indicates that the cerebrospinal fluid is not in rapid motion. About 35 minutes elapsed between the peaks of the ventricular and cisternal curves. The average distance from the foramina of Monro to the tip of the cisternal needle was 41 mm. The velocity of the fluid is therefore in the order of 1 to 2 mm. per minute. This slow apparent displacement is the result of extraneous movements, such as coughing, sighing and deep breathing, imparted to the fluid and setting up currents within it. Since it is of a much lower order of magnitude, diffusion, *per se*, can not account for this movement.

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## Influence of Adrenalin on Fibrinogen.

H. H. RIECKER AND MARY WINTERS. (Introduced by Raphael Isaacs.)

*From the Department of Internal Medicine, University of Michigan.*

Vosburg and Richards<sup>1</sup> first noted that blood coagulated more rapidly in animals after administering adrenalin, and Cannon and his coworkers reasoned from their experiments with adrenalin and from other evidence that the liver furnished some factor to increase the clotting process of the blood. In a study to determine just what this factor was, Grabfield<sup>2</sup> concluded that adrenalin decreases the coagulation time by increasing the amount of prothrombin in the circulating blood.

Grabfield could find no change in the anti-thrombin or in the fibrin content of the blood following the administration of adrenalin, but he says: "The fibrin determinations were made by the heat coagulation method of Whipple and Hurwitz, an unsatisfactory and unreliable method for this work because, chiefly, the amounts of blood required for accurate results are too large to obtain from cats without introducing the factor of hemorrhage."

In reviewing the problem it seemed that fibrin instead of prothrombin may have been an active factor in initiating the decreased coagulation time and that Grabfield's work should be repeated, using larger animals. Cannon was able to show that the increase in blood sugar concentration after adrenalin could not alone decrease the coagulation time, and since it has been shown by Whipple and Foster<sup>3</sup> that fibrin is probably stored in the liver, it was decided to study particularly this substance.

Dogs were injected subcutaneously with 1:1,000 adrenalin chloride. Of the 6 animals used, 4 were healthy and 2 had chronic secondary anemia from hemorrhage. Coagulation time was determined by a modified method of Howell<sup>4</sup> using test tubes 12 mm. in diameter. Blood sugar determinations were made by the method of Folin<sup>5</sup> and fibrin by the method of Schultz, Nicholes, Schaefer.<sup>6</sup>

We chose arbitrarily to inject, subcutaneously, 1 and 2 cc.

<sup>1</sup> Vosburg and Richards, *Am. J. Physiol.*, 1903, **9**, 35.

<sup>2</sup> Grabfield, G. P., *Am. J. Physiol.*, 1916, **42**, 46.

<sup>3</sup> Foster and Whipple, *Am. J. Physiol.*, 1922, **58**, 365.

<sup>4</sup> Howell, *Arch. Int. Med.*, 1914, **8**, 76.

<sup>5</sup> Folin, *J. Biol. Chem.*, 1929, **82**, 83.

<sup>6</sup> Schultz, Nicholes, Schaefer, *Am. J. Path.*, 1925, **1**, 101.

amounts of the adrenalin solution in dogs weighing approximately 12 kilos. This equals about 0.01 and 0.02 mg. per kilo respectively. The dogs were fasted 18 hours before the experiments were made. The blood was drawn directly into dry syringes from the iliac vessels by skin puncture in quantities not exceeding 25 cc.

The protocol shows in detail the time relations in each experiment together with the changes in fibrin and sugar content in the blood following the injection of adrenalin.

PROTOCOL.

Exp.	Time	Mg.	Fibrin per 100 cc.	% In- crease of fibrin	Blood sugar mg. per 100 cc.	Increase	Coagula- tion time, min.
I	11:11		316.0		85		4
	11:17 adrenalin 1 cc.						
II	11:32		407.6	91.6	121	29.0	2
	10:09		363.9		76		6
	10:44		324.0		76		2
	10:55 adrenalin 1 cc.						
III	11:10		482.5		85		1
	11:25		396.0	119.6	90	35.6	3
	11:07		455.7		77		6
	11:09 adrenalin 1 cc.						
IV	11:19		541.7		121		5
	11:29		540.8	85.1	113	18.7	4
	9:00 (arterial)		354.0		62		6
	10:10		320.8		62		2½
V	10:15 adrenalin 1 cc.						
	10:30		403.1		85		1½
	2:50 (highly excited)		421.6	100.8	108	31.4	2½
	10:52		343.0		77		7
VI	10:55 adrenalin 1 cc.						
	11:10		398.0		133		3
	2:06		311.2	55.0	109	16.0	6
	Anemic dog, Hb 55%						
VII	1:46		242.4		80		3½
	1:47 adrenalin 2 cc.						
	1:57		290.5		87		3
	2:03.5		365.0		91		1
VIII	2:11		346.5	122.0	91	50.5	1
	Anemic dog, Hb. 58%						
	1:56		368.6		83		2½
	1:57 adrenalin 2 cc.						
VIII	2:09		439.2		86		2
	2:14		512.7		93		1½
	2:24		553.5	194.9	95	52.9	1½
	Anemic dog, Hb. 60%						
	11:22 A. M.		306.7		101		7
	1:23.5 P. M.		303.9		97.6		7
	1:25 adrenalin 2 cc.						
	1:39		470.4		116		4
	1:56.5		478.4		150		2
	2:12.5 (arterial)		438.1	171.7	161	55.9	4

Unless indicated otherwise, venous blood was used.

The fasting fibrin levels averaged 334.3 mg. from 8 determinations, the average maximum increase after adrenalin was 36.3% when the blood was analyzed between 15 and 30 minutes following the injection.

The blood sugar determinations were made as a check upon the action of adrenalin, and are included in the protocol for this reason. There was no definite relationship between the rise in blood sugar and the decrease in coagulation time following the use of adrenalin.

The coagulation time decreased, averaging about 60%, and in each instance the decrease was definite. There was a consistent relation between the height of the fibrin values and the decrease in coagulation time, as can be seen from the protocols. We could detect no difference in results by the use of 1 or 2 cc. of adrenalin solution.

The blood fibrin increased at 10 minutes after the injection but the maximum values appeared somewhat later, and followed fairly consistently the rise in blood sugar.

In several experiments the animal was anesthetized with amytal, but this precaution to avoid the element of excitement did not affect the results. Except in one instance the dogs were accustomed to the procedure in the experiments reported.

The experiment was performed upon 2 normal human subjects using 0.6 cc. adrenalin subcutaneously. There was a marked increase in the blood fibrin. (Table I.)

TABLE I.

Showing response to adrenalin in 2 human subjects at end of 10 and 15 minutes.

	Time	Fibrin	Blood Sugar	Blood Pressure
Case A	8:25 A. M.	333.0	93.4	110/80
	8:26 Adrenalin 0.6 cc.			
Case B	8:37	437.7	123.8	140/100
	8:30 A. M.	227.0	93.5	130/80
	8:31 Adrenalin 0.6 cc.			
	8:38	261.0		180/90
	8:45	468.6	168.8	150/85

It was established in Cannon's experiments that when the abdominal viscera were excluded from the circulation, the clotting process was not hastened by the injection of adrenalin. Foster and Whipple<sup>3</sup> apparently have demonstrated that fibrin comes from the liver. However, it cannot be stated positively as they suspected that fibrin is made in the liver. Our experiments seem to demonstrate that fibrinogen at least is stored in the liver and that the action of adrenalin is to cause its quick mobilization into the blood stream.

Its action would be analogous in this respect to the increase of sugar in the blood stream following the administration of adrenalin.

After adrenalin injections the fibrin values remain high for several hours, and probably would have to be considered in interpreting nitrogen metabolism studies such as have been reported recently by Watkins and Smith.<sup>7</sup>

## 5476

## Oxidation of Cobaltous Cysteine.

EDWARD C. KENDALL AND JAMES E. HOLST.

*From the Division of Chemistry, The Mayo Foundation, Rochester, Minnesota.*

Michaelis and Barron<sup>1</sup> and Michaelis and Yamaguchi<sup>2</sup> have shown that cobalt reacts with cysteine to form a product which has a high reduction intensity. They also showed that this reaction product can be oxidized with air, ferri-cyanide, and phenolindophenol to a brown cobaltic cysteine complex. We have found that cobaltous cysteine reacts in a different manner toward each one of these oxidants. Indigo disulfonate produces a quantitative conversion of cobaltous cysteine to the cobaltic cysteine complex. All other oxidants except those which contain a quinone group result in the formation of the cobaltic cysteine complex and cystine in different proportions.

Oxidation with ferricyanide produces a unique type of electro-metric titration curve because all of the cobaltous cysteine is removed from solution by addition of a half of the total amount of oxidant. The last half of the curve represents oxidation of cysteine to cystine.

Cysteine reacts with quinone and with the quinone group of dibromophenol-indophenol with the formation of an addition product between the dye and the thiol group similar to the addition of an aromatic thiol group to quinone. One molecule of cysteine reacts with 1 molecule, that is, 2 equivalents of the dye. Ninety per cent of cobaltous cysteine is oxidized to the cobaltic cysteine complex with dibromophenolindophenol, and 10% of the cysteine combines with the dye. The amounts of cobaltic complex and cystine

<sup>7</sup> Watkins, O., and Smith, George Van S., *Am. J. Physiol.*, 1931, **96**, 28.

<sup>1</sup> Michaelis, L., and Barron, E. S. C., *J. Biol. Chem.*, 1929, **83**, 191.

<sup>2</sup> Michaelis, L., and Yamaguchi, S., *J. Biol. Chem.*, 1929, **83**, 367.

formed by each oxidant are such that the amounts of ferricyanide, oxygen, and indophenol required for a given amount of cysteine are all equal to two-thirds the amount of the cysteine.

The conversion of cobaltous cysteine into the cobaltic cysteine complex is not a straight forward oxidation such as occurs in the oxidation of cobaltocyanide to cobalticyanide. Slight variations in the velocity of addition of the oxidizing agent bring about significant variations in the proportions of cobaltic cysteine complex and cystine which are formed. The cobaltous ion is not oxidized to the cobaltic state at pH 7.4 by any of the oxidants which have been used. The results indicate that in the presence of the thiol group the cobalt is oxidized to cobaltic tricysteine. This is not stable and rearranges into a cobaltic dicysteine complex which is the brown complex described by Michaelis and coworkers. The rearrangement of cobaltic tricysteine to the complex results in the liberation of a negatively charged  $-SR$  group.

The quantitative relations in the oxidation of cobaltous cysteine with the different oxidants can be explained by variations in the relative velocities of 4 reactions:

1. Oxidation of cobaltous cysteine to cobaltic tricysteine.
2. Conversion of cobaltic tricysteine into the cobaltic dicysteine complex.
3. Oxidation of the  $-SR$  group to cystine or addition to the quinone group of a dye.
4. Recombination of the  $-SR$  group with cobalt ion.

Cystine, oxidized glutathione, sodium hydrosulfite, and sodium sulfite convert cobaltous cysteine into a cobaltic cysteine complex. Sodium thiosulfate has no effect on cobaltous cysteine. Glutathione will not form a complex with cobalt.

Formaldehyde, acetaldehyde, heptaldehyde, cyanide, and ferrocyanide decompose cobaltous cysteine and prevent its oxidation to the cobaltic complex.

Although indigo disulfonate cannot oxidize cysteine to cystine a small percentage of the total cysteine is converted into cystine with this dye if cobalt is present in less than half the molar concentration of the cysteine. This indicates activation of the thiol group which may be explained by the presence in solution of a negatively charged  $-SR$  group during oxidation of cobaltous cysteine.

5477

## On the Assaying of the Potency of Liver Extract.

J. P. MCGOWAN. (Introduced by J. J. R. MacLeod.)

*From Rowett Research Institute, Aberdeen, Scotland.*

In a previous paper<sup>1</sup> a method was described for determining in a qualitative fashion the efficacy of liver extracts. It consisted essentially in feeding varying quantities of the extract to fowls suffering from a naturally occurring disease, which resembles very closely pernicious anemia of human beings.

The procedure has since been elaborated so as to put it more or less on a quantitative footing. Liver extract does not seem to be well absorbed from the alimentary tract of fowls, and resort has been made to intraperitoneal injection with the result that there has been a very considerable lowering of the dose necessary to produce a "response" or indicator for purposes of standardization.

The procedure for standardization consists of the injection intraperitoneally twice daily over a period of time of increasing small doses of the extract until a dose is reached at which a "response" is obtained. This "response" consists in a definite rise in the hemoglobin and red blood cell curves, appearing sharply and for the first time.

The "response" for the same extract sample appears to be obtained at the same dosage, whatever may be the state of the marrow, as regards hyperplasia, etc., provided the marrow is in a condition of hyperplasia and has not become aplastic.

The "response" dose for fowls of the White Leghorn breed of about 1500 gm. weight and kept under fairly ordinary laboratory conditions is in the neighborhood of 0.225 gm. per day of the extracts employed. These were different samples of Extract No. 343, prepared by Eli Lilly & Co., Indianapolis.

At times there is difficulty in procuring experimental fowls. It is practically certain that the majority of cases of pernicious anemia in the fowl are due to infestation with the tape worm, *Davainea proglottina*. It is suggested that the supply of cases might be increased by causing fowls, known to be hosts of *Davainea proglottina*, to cohabit in close quarters in a confined area with garden slugs, the intermediate host of the tape worm. Care would have to be taken that optimum conditions in regard to dampness, vegetation,

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<sup>1</sup> McGowan, J. P., *Edin. Med. J.*, 1930, **27**, 330.

shelter, etc. for the welfare of the slugs prevailed. Under such circumstances one would expect heavier infestations and an increased supply of anemia cases.

I wish to express my great indebtedness to Dr. Clowes, Director of Eli Lilly Laboratories, for supplies of liver extract and other much appreciated favors.

## 5478

## Induction of Ovulation in Frogs and Toads.

A. ELIZABETH ADAMS.

*From the Department of Animal Genetics, University of Edinburgh,\* and Zoology Department, Mount Holyoke College.*

Experiments on the induction of ovulation in frogs and toads by means of pituitary inoculations and injections of extracts were carried on during the fall of 1930. The results obtained are in essential agreement with those of Houssay, Giusti, and Lascano-Gonzalez<sup>1</sup> and of Kehl<sup>2</sup> on different species of toads and frogs. At the same time it appears possible to explain the seemingly contradictory results in the work of these authors and in the present work without employing the idea of a "zoological specificity" of anterior lobe hormones suggested by Houssay *et al.*

Mature toads (*Bufo vulgaris*) and frogs (*Rana vulgaris*) were inoculated with anterior lobes of pituitaries or injected with extracts† of mammalian pituitary, human placenta, pregnant urine, or with 0.7% NaCl solution. The pituitaries were placed in the dorsal lymph sacs of the animals, while extracts were injected into the dorsal lymph sac, or the muscles of the hind leg, or (rarely) into the body cavity.

*Inoculations of pituitaries into toads and frogs.* I. The first series of experiments consisted of toads inoculated with toad pituitaries (Table 1).

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\* I wish to thank Professor F. A. E. Crew, Director of the Animal Breeding Research Department, University of Edinburgh, for the hospitality of the Department Laboratory during the fall of 1930.

<sup>1</sup> Houssay, B. A., Giusti, L., et Lascano-Gonzalez, J.-M., *Compt. Rend. Soc. Biol.*, 1929, **102**, 864.

<sup>2</sup> Kehl, R., *Compt. Rend. Soc. Biol.*, 1930, **103**, 744.

† These extracts were prepared by Dr. Wiesner and members of his section of the Animal Breeding Research Department, to whom I wish to express my appreciation.

TABLE I.  
Toads as hosts of toad pituitaries.

Animal	Pituitary inoculations	Period (in days) of inoculations	Findings*
BvT 1	1 whole pit. 3 pars ant.	12	2001 (or more) eggs laid on 12th through 16th day. Autopsy, 49th day: 7 eggs in oviducts, mass of degenerating eggs in uteri.
BvT 3	3 " "	10	1879 eggs laid on 14th and 15th days. Autopsy, 15th day: 14 eggs in oviducts, 9 in body cavity.
BvT 4	6 " "	10	Death from infection. (No egg-laying or ovulation.)
BvT 5	4 " "	3	1463 eggs laid on 4th and 5th days. Autopsy, 8th day: 1 egg in oviduct, none in body cavity.
BT ♀ 1	10 " "	12	1989 eggs laid on 13th day. Autopsy, 42nd day: no eggs in oviducts or in body cavity.

\* In findings, number of day is given in relation to first inoculation.

Of 5 animals receiving toad pituitary inoculations, 4 laid eggs (Table I), demonstrating the potency of the gonad-stimulating hormones of the anterior lobes. The 5th animal died of an infection. The egg-laying occurred without clasping of females by males in 3 cases. In the one instance in which a pair was treated simultaneously, the male clasped the female (BT ♀ 1) on the 4th day after the treatment had begun, continued his hold during inoculations, and only released her after the eggs had been laid. Many of these eggs were fertilized and a considerable number of embryos developed.

II. The second series of experiments consisted of toads inoculated with frog pituitaries or with frog pituitaries followed by toad pituitaries. Frog pituitaries alone did not induce ovulation or egg-laying in toads. This is definitely true in 2 cases each of which received 25 pars anterior in 41 days and as far as actual deposition of eggs is concerned it is so for 2 others (27 pars anterior in 30 days; 19 pars anterior in 30 days). It seems reasonable, though not provable, to believe that ovulation did not occur in either of these instances until toad pituitary inoculations were begun, 3 and 5 days after cessation of frog inoculations. Then 5 pars anterior of toads given in 4 days induced egg-laying in one case, while 6 in 7 days induced ovulation in the other though actual egg-laying did not occur. The ability of the toad pituitaries in relatively small numbers to induce ovulation in toads presents a marked contrast to the ineffectiveness of even large numbers of frog pituitaries.

III. The third series of experiments consisted of 5 toads inoculated with frozen pituitaries of cattle and 1 with whole pituitaries of freshly killed rabbits over periods of 15 to 23 days. Neither pars anterior alone (9, 10, 11 pieces given in periods of 21, 23, 23 days), nor pars anterior followed by posterior lobe (3 pieces of the former, 7 of the latter in 15 days), nor whole gland (5 pituitaries of rabbits in 16 days) caused ovulation in toads. It might be argued that the frozen pituitaries had lost their potency but this possibility is refuted by the fact that at the time of these experiments potent extracts (tested on mice by members of Dr. Wiesner's section) were being made from such glands.

IV. The fourth series of experiments consisted of frogs inoculated with frog pituitaries, frog muscle, or toad pituitaries (Table II).

TABLE II.  
Frogs as hosts of frog pituitaries or frog muscle or toad pituitaries.

Animal	Treatment	Findings*
F ♀ 1	13 pars ant. ( <i>R. vulgaris</i> ) in 17 days	On 6th day (6 pars ant.) some eggs squeezed from cloaca. On 13th day (8 pars ant.), uterine regions dark through body wall. On 16th day (11 pars ant.), 71 (or more) eggs pressed from cloaca. On 19th and 20th days (13 pars ant.), large clumps of eggs (1812) in water. Some eggs were fertilized by ♂ receiving inoculations and developed into embryos. Autopsy, 20th day: few eggs in uteri and body cavity.
F ♀ 3	6 pars ant.	On 5th day (4 pars ant.) uteri dark through body wall.
F ♀ 4	( <i>R. vulgaris</i> ) in 6 days	No egg-laying. Autopsy, 14th day: uteri full of eggs, some eggs in oviducts, 5 in body cavity of F ♀ 3, 13 in that of F ♀ 4.
F ♀ 5	6 muscle	No egg-laying. Autopsy, 11th day: no ovulation.
F ♀ 6	( <i>R. vulgaris</i> ) in 6 days	
F ♀ 2	13 pars ant. ( <i>B. vulgaris</i> ) in 15 days	On 15th day (11 pars ant.), uteri dark through body wall. On 19th day, large mass of eggs (1415) in water. Autopsy, 19th day: both uteri full of eggs, few in oviducts, none in body cavity.
F ♀ 11	11 pars ant. ( <i>B. vulgaris</i> ) in 6 days	On 4th day (5 pars ant.), uteri dark through body wall. No egg-laying. Autopsy, 9th day: uteri full of eggs, none in oviducts, 1 in body cavity.
F ♀ 12	10 pars ant. ( <i>B. vulgaris</i> ) in 5 days	On 2nd day (4 pars ant.), uterine region somewhat swollen. On 4th day (6 pars ant.), uteri dark through body wall. No egg-laying. Autopsy, 8th day: uteri full of eggs, few eggs in oviducts, 5 in body cavity.

\* In findings, number of day is given in relation to first inoculation.

Inoculations of pars anterior of frogs and toads induced ovulation in frogs (6 cases; egg-laying in 2 of these) while muscle was negative in its effects (Table II). The reaction after toad pituitaries is particularly interesting in that frog pituitaries are ineffective in toads.

*Experiments with injections of extracts or of salt solution.* Toads and frogs were given injections of the following substances: (1) An acid extract of human placenta (known as E94, Wiesner<sup>3</sup>) which induces cornification of the vaginal epithelium when injected into immature mice; (2) an aqueous alkaline extract of anterior lobes of cattle pituitaries (known as 119A, Wiesner and Crew,<sup>4</sup> resembling Evans's aqueous alkaline extract) which induces mucification of the vaginal epithelium when injected into immature mice; (3) 0.7% NaCl solution. In addition toads received injections of an extract made from pregnant human urine (known as 198E, Marshall<sup>5</sup>) which induces formation of blood spots and corpora lutea in the ovaries when injected into immature mice.

I. The first series consisted of injections of E94, 119A, and 0.7% NaCl into toads and frogs. E94 in toads (11.25 cc. in 14 days; 43 cc. in 46 days; 57 cc. in 60 days) or in frogs (9 cc. in 5 days; 12.3 cc. in 9 days), 119A in toads (16.65 cc. in 38 days; 17.5 cc. in 20 days; 27.5 cc. in 59 days), 0.7% NaCl in toads (51, 52, 60, 60, 60 cc. in 56, 57, 60, 60, 60 days respectively) or in frogs (9 cc. in 5 days; 12.3 cc. in 9 days) did not cause ovulation. (In 2 toads only immature tissue was found at autopsy.) *But* injections of 119A into frogs (9 cc. in 5 days; 12.3 cc. in 9 days) did induce ovulation. In these 2 cases no egg-laying occurred but that may have been due to the relatively short period of the experiment.

II. The second series consisted of injections of 198E, and of combinations of E94, 119A, 198E, and inoculation of frog pituitaries into toads. No type of injection, *i. e.*, of a single extract (198E: 6 and 8.8 cc. in 28 and 31 days respectively) or of various combinations of extracts (E94 with 119A: 15, 46, 10 cc. of the former with 21.95, 11, 26.65 of the latter in 59, 60, and 61 days respectively; 119A with 198E: 11, 19.5, 27.2 cc. of the former with 0.5, 4.5, 4.5 cc. of the latter in 25, 42, and 58 days respectively) or of extracts with frog pituitary inoculations (15 cc. of E94 with 17.05 cc. of 119A plus 10 pars anterior in 59 days) induced egg-laying or ovulation in toads. Up to the present similar series of experiments have not been carried out on frogs.

These results seem to permit the following interpretation. The pars anterior of frog and toad pituitaries and aqueous alkaline extracts of anterior lobes of mammalian pituitaries contain hormones capable of stimulating frog ovaries to release their eggs, but only the

<sup>3</sup> Wiesner, B. P., *Edinburgh Med. J.*, February, 1930.

<sup>4</sup> Wiesner, B. P., and Crew, F. A. E., *Proc. Roy. Soc. Edinburgh*, 1929, **50**, 79.

<sup>5</sup> Marshall, unpublished data.

hormones of the toad cause this reaction in toads. The statement is supported by the facts that ovulation occurred in toads after toad anterior pituitary inoculations (*cf.* Houssay *et al*<sup>1</sup> in toads, *B. arena-rum* and *B. d'Orbigny*) and in frogs (1) after frog pituitary inoculations (*cf.* Wolf<sup>6</sup> in *R. pipiens*), (2) after toad pituitary inoculations (*cf.* Houssay *et al*), and (3) after injections of an aqueous alkaline extract of mammalian anterior lobes (*cf.* Kehl<sup>2</sup> in *Discoglossus pictus*). The negative results in toads after inoculation with frog and mammalian (cattle, rabbit) pituitaries agree with those of Houssay *et al*, who also report negative findings after implants of fish, frog, snake, hen, rat, guinea-pig, dog and beef pituitaries. These authors interpret their results as indicative of a "zoological specificity" of the anterior lobe hormone but to the writer it seems rather to indicate either a lack of sensitivity to potent hormones or a neutralization of these hormones by the body fluids or secretions of the toad. On the other hand, the absence of any reaction on the part of both toads and frogs to placental extracts (E94) argues against the presence of ovulation-inducing hormones in this extract. The salt solution injections merely serve as controls in experimental procedure and indicate that the shock of injecting extraneous fluids will not bring about ovulation. The negative results after injections into toads of the extract made from pregnant urine (198E; *cf.* Houssay *et al*), should be checked by similar experiments on frogs before a lack of potent hormones can be asserted.

Thus on the basis of the present experiments in frogs and toads as well as those of Wolf, Houssay *et al*, and Kehl, and the evidence that urodeles respond to anterior lobe hormones of anurans (ovulation occurs in *Triturus viridescens* after implants of pars anterior of the frog, *R. pipiens*, Adams,<sup>7</sup> and of the toad, *B. terrestris*, Adams<sup>8</sup>) as well as to those of urodeles (Noble and Richards,<sup>9</sup> Adams<sup>10</sup>), the tentative hypothesis is offered that the apparently contradictory results secured in frogs and toads can be better explained as due to a peculiarity of females of the genus *Bufo* in failing to react to gonad-stimulating hormones of pituitaries other than their own rather than to a "zoological specificity" of these hormones. This hypothesis is to be tested by further experimentation.

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<sup>6</sup> Wolf, O. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 692; *Anat. Rec.*, 1929, **44**, 206.

<sup>7</sup> Adams, A. E., *Anat. Rec.*, 1930, **45**, 250.

<sup>8</sup> Adams, A. E., *Anat. Rec.*, March, 1931.

<sup>9</sup> Noble, G. K., and Richards, L. B., *Am. Mus. Novitates*, 1930, Jan. 9, No. 396; *Anat. Rec.*, 1930, **45**, 274.

<sup>10</sup> Adams, A. E., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 433.

### Effects of Frequency and Intensity in Stimulation of Cervical Sympathetic Nerve Fibers to Eye.

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Gruber<sup>1</sup> has shown that in the results of both afferent and efferent stimulations, in some instances essentially the same effect is produced by a high frequency and weak intensity of stimulation as by a low frequency of higher intensity, while in other instances an opposite result occurs. In afferent stimulation, spatial and temporal summation might account for the phenomena, which, however, offer more difficulty of interpretation when direct stimulation of efferent nerves show a similar effect. Dr. Gruber suggested that we attempt to correlate his findings with the analysis of fiber types which we have been carrying on by means of the oscillograph.

Results of stimulation of the cervical sympathetic were employed as criteria: dilatation of the pupil and retraction of the nictitating membrane of cat and rabbit; vasoconstriction in the conjunctiva of the cat, and in the ear of the rabbit; and pilometer effects upon the hair between ear and eye of the cat. The nerve was stimulated posterior to the superior ganglion (mostly preganglionic fibers) and observations were made on the peripheral response in the animal, decerebrated or anesthetized, while action potentials were being recorded from the cut central end of the nerve. For the most irritable fibers giving rise to the first wave of the cervical sympathetic action potential picture, no effect can be detected from peripheral stimulation, and these fibers have previously been judged to be afferents (Bishop and Heinbecker<sup>2</sup>). The second potential component is double, and consists of the main potential of this nerve, arising from small myelinated sympathetic fibers. Stimulation of the first division of this group, giving a distinct wave in the potential picture, elicits the effect on the pupil and nictitating membrane of the eye. Stimulation of the second group elicits the pressor effect and the pilomotor effect. No effect has yet been detected from stimulation of the last group, of unmyelinated axons, and this, together with the effect of stimulation of the very short postganglionic stretch available in this nerve, is being further investigated.

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<sup>1</sup> Gruber, C., *Am. J. Physiol.*, 1915, **36**, 299; **37**, 259.

<sup>2</sup> Bishop, G. H., and Heinbecker, P., *Am. J. Physiol.*, 1930, **94**, 170.

It can be stated at present that there is no repetition postganglionically from single stimuli preganglionically, and that the amplitudes of potential, pre- and postganglionic, are closely proportional as stimulus strength is increased. Therefore, spread and summation do not take place in this ganglion, where most if not all of the efferent fibers here concerned show a synaptic delay.

In all these effects, so far as could be judged, the same area could be affected to the same degree by weak and rapid as by strong and slow stimulation. When the action potentials were examined, however, it was found in every case that none of a given effect was produced without a strength of stimulation that excited the *most irritable fibers* of a *specific group*, as indicated by a wave in the potential record. Further, no increase of a given effect was produced at a given rate of stimulation by increasing the stimulus strength *from the maximum* to beyond the maximum of that group. The least irritable fibers of a given group may have a threshold at least 2 or 3 times that of the most irritable, the most irritable fibers of the 2 main groups here studied having a threshold ratio of about 1 to 4. Within the group concerned, a single maximum stimulus is effective for certain of the effects, but does not give the maximal response obtainable by repeated maximal stimuli. The number of fiber-stimuli rather than frequency or intensity alone thus determines the response as a whole. The fibers in the nerve responsible for each effect are thus distributed over a specific range, limited as to threshold and conduction rate.

Some peripheral mechanism appears to exist for distributing the effect of the stimulation of a few fibers over the whole area that is capable of being affected by the rest of the fibers of higher threshold in that group. This may be simply a spatial distribution in the peripheral tissue, only a few scattered muscle fibers responding to a "threshold" stimulus, but the magnitude of the total response from repeated stimulation of a few nerve fibers suggests the possibility of a peripheral summing mechanism, such as there appears to be in the heart (Heinbecker<sup>3</sup>). Conduction from one smooth-muscle fiber to another directly, upon repeated stimulation of a few fibers, or spread by way of a peripheral nerve-network, is not perhaps excluded.

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<sup>3</sup> Heinbecker, P., *Am. J. Physiol.*, 1930, **93**, 656.

## 5480

**Comparison of the Reducing Power of Cancer Tumors and Tumors Produced by Filterable Viruses.**

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RAYMOND E. GARDNER.

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There are in the literature contradictory reports with regard to the reducing power of tumor tissues. While Drew<sup>1</sup> and Heinlein<sup>2</sup> speak of a diminution of the reducing power of malignant tumors when compared to normal tissues, Voegtlin, Johnson and Dyer<sup>3</sup> maintain that such diminished power does not exist. Yaoi and Nakahara,<sup>4</sup> working with Rous chicken sarcoma, report that while this tumor when heated to 56° for half an hour and then incubated at 37°C. under a layer of vaseline, is unable to reduce methylene blue in the presence of sodium succinate, skeletal muscle submitted to the same treatment reduces the dye in 16 hours.

We have studied the reducing power of tumor tissues using the following technique. The tissue was cut in a manner similar to the Warburg technique for tissue respiration. This was placed in M/15 Sorensen's phosphates pH 7.38. Methylene blue was mostly used as indicators of reduction intensity. A stream of purified nitrogen was passed through the tubes. This kept the tissues in continuous movement throughout the entire column of liquid and facilitated the reduction of the dye. Rubber connections were entirely eliminated from the system. The tubes were kept in an air bath at 37.5°C.±0.3.

Seven different strains of rat's malignant tumors and one rabbit tumor have been studied. Our results show that tumor tissues have practically the same reducing power as normal tissues. When an easily oxidizable substrate (in our experiments sodium succinate) is added to the medium, the time of reduction is shortened, thus showing in all of these tumors the presence of the enzyme (succino-dehydrogenase) which activates biological oxidation-reduction systems.

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<sup>1</sup> Drew, A. H., *Brit. J. Exp. Path.*, 1920, **1**, 115.

<sup>2</sup> Heinlein, H., *Z. f. Krebsforschung*, 1930, **30**, 506.

<sup>3</sup> Voegtlin, C., Johnson, J. M., and Dyer, H. A., *J. Pharm. and Exp. Therap.*, 1925, **24**, 305.

<sup>4</sup> Yaoi and Nakahara, *Proc. Imp. Acad.*, 1927, **3**, 102.

TABLE I.

Tissue	Time of complete reduction of methylene blue in minutes	
	With Na succinate	Without Na succinate
Rat Walker round cell sarcoma No. 319.	9	14
" " medullary carcinoma No. 256.	14	20
" " spindle cell carcinoma No. 155	25	30
" " carcinoma No. 2	10	15
" " adenocarcinoma No. 12	9	14
" " benign breast adenofibroma	17	22
" Crocker small spindle cell sarcoma	10	—
Rabbit epithelioma	23	—
Rous chicken sarcoma	114	114
Chicken muscle	14	24
Rabbit testicle—normal	23	—
" " virus III	36	—
" " neurovaccine	63	83
" brain—herpes	29	37
" " normal	14	19
Fowl— <i>Epithelioma contagiosum</i> (eye)	52	90
" " " (skin)	47	—
" normal skin	47	60
Rabbit myxoma	no reduction	no reduction

Tumors produced by filterable viruses show a striking contrast in their behavior towards the reduction of reversible dyes when compared to malignant tumors. Rous chicken sarcoma and rabbit myxoma have been studied. In addition to these tumors we have studied the reducing power of tissues where some filterable viruses were growing, namely, herpetic encephalitis (brain) rabbit virus III (testicle), neurovaccine Levaditi (rabbit testicle), and *epithelioma contagiosum* (fowl's eye and skin). As can be seen in Table I, both rabbit myxoma and Rous chicken sarcoma lack succinodehydrogenase and at the same time show lower reduction power than malignant tumors. Rabbit myxoma at the end of 5 hours reduces only 40% of 2-6-dichlorophenolindophenol (which would mean an  $E_o$  of +0.158 volts). Tissues where filterable viruses are grown, show also a diminished power of reduction when compared to normal tissues, with the exception of *epithelioma contagiosum*. In this particular case there was a great amount of normal skin. Succinodehydrogenase was still present although there is a partial inhibition of the enzyme as shown by a slower rate of reduction.

5481

**Dissociation of Avian Tubercle Bacilli.\***

ELEANOR G. ALEXANDER. (Introduced by R. L. Kahn.)

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This is a preliminary report of an attempt to dissociate avian tubercle bacilli by growing the organisms on a medium containing specific antiserum. The organisms were obtained from Michigan State College at East Lansing and were grown on slants on Long's agar medium. The growth was found to be orange colored, rough and corrugated and the organisms were considered to be of the R type.

The antiserum was obtained by injecting 2 rabbits with emulsions of these organisms in sterile physiologic salt solution containing a concentration of 1 mgm. per 1 cc. The emulsions were heated for 45 minutes at 56°C. before the injections. Each rabbit received a total of 9 injections of 1 cc. every 3 to 4 days and the last one consisted of 2 cc. Two weeks after this injection, the serum of each of these rabbits was tested for complement fixing substances using a suspension of avian bacilli heated for one hour at 60°C. Both sera gave strongly positive complement fixation reactions against sera of non-inoculated rabbits which gave negative results. A week later both rabbits were exsanguinated and the sera obtained under sterile conditions.

Transfers from corrugated growth of the avian bacilli on Long's agar slants were made on similar slants containing 10% of the antiserum. After about 4 weeks' growth 2 types of tubercle bacilli were observed: a preponderance of small, smooth, round, moist, white colonies having an average size of streptococcus colonies corresponding to the S type, and some typical, rough, corrugated, orange colored R colonies. Control agar slants containing, respectively, 10% normal serum and no serum showed only a few smooth colonies and consisted of R type of growth.

These results indicate that the presence of anti R serum is capable of dissociating avian tubercle bacilli R type into S. The S type may possibly correspond to certain colonies obtained by Petroff,<sup>1</sup> with an avian strain, grown on his medium, which he described as moist and round and which he calls S.

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\* This study was aided by a grant from the Commonwealth Fund.

<sup>1</sup> Petroff, S. A., and Steenken, W., Jr., *J. Exp. Med.*, 1930, **51**, 831.

### Acute Toxicity of Some Mercurial Compounds for the Circulation on Intravenous Injection.

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From the toxicological point of view, the 2 most important organs undergoing pathological changes in mercurial poisoning are the kidneys and the intestines, both exhibiting severe inflammatory and degenerative changes. It is well known, however, that the mercury ion produces a depressant effect on the circulation. According to Sollmann,<sup>1</sup> all metals produce a very marked fall of blood pressure, which is partly due to the paralysis of the blood vessels and partly to a direct action on the heart. The metals, for this purpose, may be divided into 2 groups, one group acting mainly peripherally on the blood vessels and the other group acting mainly peripherally on the heart. Mercury belongs to the latter group.

The writer has studied for some time the toxic effects on the circulation, and especially on the heart, of continual intravenous injections of various mercurial compounds, and the results, of considerable toxicological and practical interest, are here reported. The method of experimentation was simple. All the experiments were performed on cats and the technique followed was similar to that employed in the assay of digitalis by the Hatcher-Brodie cat method. The animals are kept under light ether anesthesia and a solution of a given mercurial is injected into the femoral vein from a burette at regular intervals, usually at the rate of 1 cc. a minute, and the effects upon the heart are carefully observed. When the inorganic salt, mercuric chloride, or bichloride of mercury, in concentration of 1:1000, is thus injected into the vein of a cat, the depressant effect of the mercury for the heart is manifested after a few minutes. Even after the injection of a small quantity of the solution, the heart-beat becomes feeble and the heart rapidly develops a block, after which, on continued injection of the drug, it goes into fibrillation and finally stops. The author found that the lethal dose required to produce arrest of the heart by such continual injections of mercuric chloride (1:1000, at the rate of 1 cc. a minute) is 30 mg. per kilo weight of the animal. If, however, a considerable quantity of blood is first removed from the animal and this blood

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<sup>1</sup> Sollmann, T., *Manual of Pharmacology*, second edition, 1922, 856.

is replaced with an equivalent amount of physiological sodium chloride solution, the minimal lethal dose is even less, namely, about 15 mg. per kilo weight of the animal. This difference is probably due to the partial binding of the inorganic mercury by the blood proteins in case of the non-exsanguinated animal.

A solution of mercury benzoate, prepared by dissolving this compound in solution of 0.45% of sodium chloride was injected into cats by the above method and the lethal dose was also found to be very small, 38 mg. per kilo weight of cat producing arrest of the heart and death. An examination of still another inorganic compound gave quite a different result. Mercuric iodide, or "red iodide" of mercury, is practically insoluble in water but it can be dissolved in a solution containing approximately its weight of sodium iodide. When such a solution of mercuric iodide, in concentration of 1:1000, was injected into the vein of a cat, it was found to be much less toxic than the mercuric chloride or the mercury benzoate, the lethal dose being 125 mg. per kilo weight of the animal. This great difference in toxicity between the bichloride and the iodide of mercury solutions is undoubtedly due to the difference in their manner of dissociation. The bichloride dissociates, yielding mercuric ions; the "red iodide" of mercury forms a double salt with the sodium iodide and when this double salt is dissociated, it does not yield free mercuric ions to any appreciable extent. It may be well to add that sodium iodide and the iodide ion alone have been shown long ago by the author not to be depressant for heart muscle.<sup>2</sup>

A series of organic chemical compounds were also studied toxicologically in the manner described above. The compounds examined were oxi-mercuri-di-brom fluorescein, or mercurochrome-220 soluble, the di-mercury form of mercurochrome; mono-mercuri fluorescein, or flumerin; and mono-hydroxy-mercuri-di-iodo-resorcin-sulphon-phthalein, a new compound recently described by Dunning and Farinholt.<sup>3</sup> The results are exhibited in Table I. It will be noted that, when studied on the circulation, all the organic compounds were much less toxic than the inorganic compounds of mercury (with the exception of the mercuric iodide), thus indicating that the mercury in these compounds is in firmly bound organic combination and not easily broken up in the blood stream in such acute experiments as are under discussion in the present investigation. These findings are of considerable practical interest. The author has had occasion to examine various specimens of spurious

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<sup>2</sup> Macht, D. I., *Johns Hopkins Hosp. Bull.*, 1914, **25**, 278.

<sup>3</sup> Dunning, F., and Farinholt, L. H., *J. Am. Chem. Society*, 1929, **51**, 804.

substitutes for oxi-mercuri-di-brom fluorescein, or mercurochrome-220 soluble, and has found the present method valuable in detecting inorganic mercury in such solutions. Thus it will be seen from the table that when mercuric chloride, 1:5000, is added to the solution of mercurochrome, 1:200, the toxicity of such a mixture for the cat's heart is much greater than that of pure mercurochrome. Even one part to ten thousand of bichloride can also be detected in this way. This method, unfortunately, does not apply to the detection of mercuric iodide when added to mercurochrome solutions but in such cases a very simple chemical examination can readily establish the presence of an iodine compound in the preparation, on the one hand, and of inorganic mercury, on the other. Practically no difference was noted in the toxicity of the organic mercurial compounds between exsanguinated and non-exsanguinated animals.

TABLE I. *Toxicities for Cats.*

Compound	Concentration injected, 1 cc. a min.	Lethal Dose mg. per kilo
Mercuric chloride (after previous bleeding)	1:1000	15
Mercuric chloride (without previous bleeding)	1:1000	30
Mercury benzoate (in NaCl solution)	1:1000	28
Mercuric iodide ("red iodide" in NaI solution)	1:1000	125
Oxi-mercury-di-brom fluorescein (mercurochrome-220)	1:200	150
Di-mercury-mercurochrome	1:200	140
Mono-hydroxy-mercuri-di-iodo-resorcin-sulphon-phthalein (merodicein)	1:500	200
Mono-mercuri-fluorescein (flumerin)	1:200	140
Mixture of { mercurochrome mercuric chloride	1:200	{ 104 5
	1:5000	

*Summary.* 1. Continuous intravenous infusion of solutions of mercurials in cats produces depression of the heart muscle, heart block and arrest of the heart. 2. There is an enormous difference in toxicity between organic and inorganic mercury compounds in this respect, the inorganic mercurials being much more toxic owing to the free play of the mercury ion in the solutions. 3. Solutions of mercuric iodide in sodium iodide are an apparent exception to the rule because of the formation of a double salt of mercury and sodium iodide and the peculiar dissociation of this compound in solution. 4. This effect of mercurials on the circulation is useful in the detection of inorganic mercurials which may be mixed with solutions of true organic mercury compounds.

5483

### A Comparison Between Starvation and Avitaminosis on the Duration of Life.

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It is frequently stated that starved birds and mammals live longer than such birds and mammals on a vitamin-free diet.

To test the truth of such statements the following experiments were performed.

No. 1. Six pigeons 7 weeks old were obtained from a common hardy barn-yard flock, and were normal in every respect as far as could be learned. Three were placed in Cage 1 and given distilled water only; the other 3 were placed in Cage 2 and given distilled water and commercial polished rice *ad lib*.

One of the birds in Cage 1 died the 8th day, another died the 9th day; the third bird died the 10th day. Post mortem examination showed only marked absence of adipose tissue, death evidently being due to starvation.

One bird in Cage 2 (polished rice and distilled water) developed signs of *Polyneuritis gallinarum* on the 14th day. This condition advanced in severity until the 18th day, when the bird died. Another bird in Cage 2 developed polyneuritis on the 15th day and died on the 19th day.

The third bird in Cage 2 developed *Polyneuritis gallinarum* on the 15th day. The severity of the condition steadily advanced until the 18th day, when the bird was "force-fed" brewer's yeast and recovered.

No. 2. Ten albino rats one month old were put on a generous tablescrap diet until they were 120 days old. Six of these 10 rats were then put in false bottom cages on distilled water. One rat (female) died the 9th day. A second rat (female) died the 9th day. Another rat (male) died the 9th day. The fourth rat (female) died the 10th day. The fifth and sixth rats (both males) died the night of the 10th day.

Post mortem examinations showed only marked absence of adipose tissue, death evidently being due to starvation. From 37% to 41% of the original body weight had been lost by each animal.

The remaining 4 of the original 10 rats were put in false bottom cages and given distilled water and the following diet:

	%
Harris' (vitamin-free) casein	18
Harris' (vitamin-free) starch	53
Crisco (commercial)	25
Mendel's normal salt mixture	4
	<hr/> 100

After 41 days on the above diet one rat had lost 19%, another 21%, another 24% and the fourth 26% of the respective original weight. Two animals had developed a mild xerophthalmia.

## 5484

## In vitro Effects of Certain Drugs on Strongyloides.

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The success attendant on the oral administration of gentian violet and crystal violet in eradicating or greatly reducing *Strongyloides stercoralis* in the human host (de Langen,<sup>1</sup> Faust<sup>2</sup>) as well as in other primates suggested the desirability of testing *in vitro* the strongyloidicidal properties of several therapeutics. While it was not possible to secure living parasitic *Strongyloides* for this purpose, the several successive stages of the free-living generation of an indirect type of *Strongyloides fülleborni* from the red-spider monkey, *Ateles geoffroyi*, were available. These organisms had been cultured in undiluted moist feces. At the appropriate time, when active free-living males and females, rh<sub>2</sub>, RH<sub>2</sub> and f<sub>2</sub> larvae were both numerous and active, the nematodes were drawn off in a few drops of clear water by means of the Baermann technic.

In the first instance these several stages were placed in 0.1% of each of the following reagents: crystal violet (cv); acriviolet (acv); acriflavine (acf); mercurochrome (mc), and hexyl resorcinol (hr). The results of this test are shown in the accompanying table.

In view of these results, showing the high potency of hexyl resorcinol in the dilution used, the organisms were then tested in 0.01, 0.002 and 0.001% dilutions of this drug. The tests in this case showed that the 0.01 dilution was almost as potent as the 0.1 dilu-

<sup>1</sup> de Langen, C. D. 1928. Meded. v. d. Dienst d. Volksgezondheid in Ned.—Indie. 15 pp. Weltevreden.

<sup>2</sup> Faust, E. C., *Internat. Med. Digest*, 1930, 17, 57.

TABLE I.  
*The effects of certain reagents in 0.1% solution on the free-living stages of Strongyloides fülleborni.*  
 (25 or more specimens of each stage were used in this study.)

Time	Stages	cv	acv	acf	mc	hr	Controls
1 min.	♂	+	+	+	+	±	+
	♀	+	+	+	+	±	+
	rh	±	+	+	+	±	+
	RH	+	+	+	+	±	+
	f	+	+	+	+	±	+
2 "	♂	+	+	+	+	—	+
	♀	+	+	+	+	—	+
	rh	±	+	+	+	—	+
	RH	+	+	+	+	+	+
	f	+	+	+	+	+	+
5 "	♂	+	+	+	+	—	+
	♀	+	+	+	+	—	+
	rh	±	+	+	+	—	+
	RH	+	+	+	+	±	+
	f	+	+	+	+	±	+
10 "	♂	+	+	+	+	—	+
	♀	+	+	+	+	—	+
	rh	±	±	+	+	—	+
	RH	+	+	+	+	—	+
	f	+	+	+	+	—	+
20 "	♂	±	±	+	+	All stages dead	+
	♀	±	±	+	+		+
	rh	—, ±	±	±	+		+
	RH	+	+	+	+		+
	f	+	+	+	+		+
30 "	♂	—, ±	±	±	+		+
	♀	—, ±	±	±	+		+
	rh	—, ±	±	±	+		+
	RH	+	+	±	+		+
	f	+	+	±	+		+
1 hr.	♂	—	±	±	+		+
	♀	—	±	±	+		+
	rh	—	—	—	+		+
	RH	±	±	±	+		+
	f	±	±	±	+		+
12 "	♂	—	—	—	±		+
	♀	—	—	—	±		+
	rh	—	—	—	—		+
	RH	—	—	—	±		+
	f	—	—	—, ±	±		+

+ = alive and active; ± = alive but inactive; — = dead.

tion, while the 0.002 dilution required 20 minutes to kill the worms. The 0.001 dilution was not effective under 1 hour; in other words, it was about as lethal as 0.1% crystal violet.

*Conclusions.* These *in vitro* tests with the free-living stages of *Strongyloides fülleborni* indicate that crystal violet in 0.1% dilu-

tion is more lethal than acriviolet or acriflavine in the same dilution, while mercurochrome is essentially ineffectual. On the other hand hexyl resorcinol in 0.1 and 0.01 dilutions showed a much greater strongyloidicidal capacity than any of the other reagents, and in 0.001 dilution was practically as potent as crystal violet in 0.1 dilution.

Since these *in vitro* results are suggestive only and do not necessarily parallel the *in vivo* action of these reagents, there is need to determine if hexyl resorcinol and other drugs of this series are as effective as gentian violet or crystal violet in eradicating Strongyloides from normally or experimentally infected hosts.

## 5485

### Changes in the Liver Following Cholecystgastrostomy and Cholecystduodenostomy.\*

I. M. GAGE. (Introduced by Alton Ochsner.)

*From the Department of Surgery, Tulane University School of Medicine.*

The presence of severe liver damage following cholecystgastrostomy and cholecystduodenostomy in the dog has been demonstrated by Lehman,<sup>1</sup> Horsley,<sup>2</sup> Beaver,<sup>3</sup> and Gatewood and Stanley.<sup>4</sup> The changes noted have been of varying degrees, from simple lymphangitis to extensive necrosis and abscess formation. All agree that in their experiments a hepatitis of varying degrees developed following anastomosis of the gallbladder to the gastro-intestinal tract. However, these authors failed to obtain specimens of the liver for histological study with concomitant bacteriological studies of the normal liver, stomach, or duodenum in order that a comparison could be made with sections of liver removed at varying intervals after the anastomosis of the gallbladder to the stomach and duodenum.

In 1909 Walbach and Saiki<sup>5</sup> demonstrated that areas of necrosis occurred in the liver of normal dogs.

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\* Aided by a grant from the David Trautman Schwartz Research Fund.

<sup>1</sup> Lehman, E. P., *Arch. Surg.*, 1924, **9**, 16.

<sup>2</sup> Horsley, J. S., *South. Med. J.*, 1927, **20**, 669.

<sup>3</sup> Beaver, M. G., *Arch. Surg.*, 1929, **18**, 899.

<sup>4</sup> Gatewood and Stanley, *S. G. and O.*, 1930, **50**, 40.

<sup>5</sup> Wolbach, S. B., and Saiki, T., *J. Med. Research*, 1909, **21**, 279.

Previously I reported on the bacteriology of the liver before cholecystgastrostomy and cholecystduodenostomy followed by a second bacteriological check-up 15 days after the first operation. I found practically no changes in the bacterial flora of the liver after cholecystgastrostomy and cholecystduodenostomy.

This paper deals with the changes found in the liver of normal dogs before and after anastomosis of the gallbladder to the gastrointestinal tract. A series of 40 dogs were used, divided into 2 groups. In the first series of 20 dogs cholecystgastrostomy was performed in 10 and cholecystduodenostomy in the remaining 10. In the second group a plastic operation on the gallbladder was done, producing a tube leading from the fundus of the gallbladder. The tube was inserted into the stomach and duodenum by a method similar to Coffey's method of ureteral transplantation. A series of 20 dogs were also used in this series, in 10 of which the gallbladder was anastomosed to the stomach and in the remaining 10 an anastomosis of the gallbladder to the duodenum was performed. This procedure was performed for the purpose of preventing reflux from the stomach and duodenum into the biliary apparatus.

There was an immediate operative mortality of 62.5% (peritonitis) and a total mortality of 90.7%; one animal still survives. Fifteen dogs (37.5%) were subjected to a second operation in order that a specimen of liver could be obtained for histological and bacteriological studies, and comparison made with the normal liver. The livers of 4 normal puppies, 8 days old, were also studied histologically in order to determine whether or not early pathological changes occurred and whether they corresponded to the changes in normal adult dogs.

The histological study of sections removed from the livers of the 40 normal animals revealed histopathological changes in 100%. The changes noted were small areas of necrosis of liver cells with round cell infiltration. The above areas at times were rather extensive and in some instances simulated abscess formation. The round cells consisted mainly of lymphocytes; however, polymorphonuclears and eosinophiles were also present. Perivascular infiltration with round cells was of common occurrence, appearing in Glisson's capsule and also noted around the central vein. There were no changes noted in the wall or epithelial lining of the biliary ducts. In one dog, bacteria (spore-bearing gram-positive bacillus) were found in the sinusoids with associated necrosis of the surrounding liver cells. A most interesting observation was noted which may have a bearing on the focal areas of necrosis. This was the finding of filaria in

the lumen of the blood vessels and sinusoids of the liver. This interesting condition was noted in 16 sections of normal livers, an incidence of 40%.

A study of liver sections removed 15 days postoperatively from the animals (15 in number) that survived the operation and appeared normal, revealed the following histopathological changes: In 8 (53.3%) there was no increase in the inflammatory process when compared with the corresponding sections of normal liver. The remaining 7 (46.6%) revealed a slight to moderate increase in the pathological findings when compared to the corresponding normal livers. The changes noted in this group (46.6% of the total) were: increase in size of the areas of necrosis, slight dilatation of the bile ducts, increase in congestion of blood vessels, packing of red cells in the sinusoids near the central vein, and the presence of bacilli in 3 sections.

Study of sections removed from the livers of the 8-day-old puppies showed changes in 2 puppies similar to those noted in the adult dog, in the remaining 2 there were no changes that could be interpreted as pathologic. It is of interest that the 2 livers showing pathologic changes showed infection when cultured, whereas culture of the 2 normal livers was sterile.

*Conclusions.* 1. The livers of 40 normal dogs showed pathological changes in 100%. 2. The pathological changes noted in the normal livers were: lymphocytes, perivascular round cell infiltration, areas of necrosis of the liver cells with infiltration of round cells, the presence of filaria in the lumen of the hepatic blood vessels and sinusoids, and spore-bearing gram-positive bacilli among the liver cells. 3. The microscopic changes found in the liver following cholecystgastrostomy and cholecystduodenostomy showed only a moderate increase over the normal findings in only 46.6%. 4. In all experimental research regarding changes in the dog's liver following certain types of operations, it is imperative that sections of normal liver be obtained in order that a comparison can be made with the changes found postoperatively.

Attempts to Produce Encystment in *Chilomastix*.

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The life histories of mammalian intestinal flagellates, particularly with reference to the process of encystment, have been investigated with much less satisfactory results than have those of the intestinal amoebae. Practically nothing is known regarding the conditions producing encystment or excystment in these forms. The results here reported are largely negative, but it seems worth while to summarize them briefly since they show what conditions are incapable of producing encystment *in vitro*, and since, furthermore, this phase of the investigation is being abandoned.

Da Cunha and Muniz<sup>1</sup> are the only workers to report encystment of *Chilomastix mesnili* in culture; their successful results were obtained with ordinary blood agar and with N.N.N. medium, on which the flagellates encysted in great numbers after being grown for 48 hours at 37°C. Experimental production of encystment in *C. intestinalis* from the guinea pig has been reported by Hegner.<sup>2</sup> Washed cysts from guinea pigs were fed young chicks and apparently hatched in the digestive tract of the latter, since motile forms were discharged in cecal droppings 6 days later. Four days after this, cysts as well as trophozoites were abundant in the cecal droppings, but by the fourteenth day the infection had disappeared. The chick, in this instance, served as a living culture tube.

In a series of experiments running over several years, *Chilomastix mesnili* was cultivated successfully in a number of standard culture media, such as ovo-mucoid, Ringer-egg (with and without albumen), Locke-egg (with and without albumen), and Locke-egg-serum. In cultures continued over several months, there was no evidence of anything approaching a life cycle, such as any rhythmic variation in the division-rate; and no cysts were ever found. On Locke-egg-albumen, the flagellates appear able to grow and multiply indefinitely without producing cysts.

It was not possible to produce encystment of motile *Chilomastix* *in vitro*, despite numerous attempts to obtain it by modification of

\* Aided by a grant from The Board of Research of the University of California.

<sup>1</sup> Da Cunha, A. M., and Muniz, J., *C. E. Soc. Biol.*, 1927, **97**, 1777.

<sup>2</sup> Hegner, R., *Am. J. Hyg.*, 1929, **9**, 529; **10**, 33.

the above cultural conditions. The following modifications of the standard culture media were made with the aim of producing encystment. The viscosity of the medium was increased by gradual evaporation or by the addition of dilute agar, gelatin or gum arabic. The concentration of substances normally present in the feces was increased without producing an unfavorable preponderance of bacteria by the addition of purified indol or skatol. Purified cholesterol was also employed. Both sterile rice starch and sterile rice flour were added to the various culture media. Although ingested by the trophozoites, they did not bring about any marked increase in their numbers nor cause them to encyst. The results of da Cunha and Muniz could not be confirmed; N.N.N. medium did not prove satisfactory for the cultivation of *Chilomastix intestinalis* of the guinea pig, even when guinea pig blood was used in its preparation. Care must be taken to avoid the inoculation of cysts into this medium, lest the process be supposed to take place in the culture tube. Lastly, the cultures were brought to room temperature, either gradually, or abruptly, as would be the fate of organisms expelled from the digestive tract, with again negative results.

Hegner's results have been amply confirmed in the transfer of mammalian *Chilomastix* to young chicks. Rectal injections of motile trophozoites of *C. mesnili* and of *C. intestinalis* have been made into 126 young chicks, for the most part 24 to 48 hours old. In 39 of these, infection was established and maintained for 24 hours or more; chicks frequently continued to discharge trophozoites for at least 4 days; the longest period for which they remained infected was 8 days. The chick cecum, for a time at least, offers a particularly favorable environment for *Chilomastix*, these flagellates were found frequently in relatively greater numbers in the chick than in the original host. Conditions in the chick cecum are apparently more suitable for *Chilomastix* than for the Protozoa associated with it in the guinea pig; *e. g.*, *Trichomonas* or *Balantidium*, since inoculation of material from the guinea pig cecum into the chick often results in the partial or complete disappearance of these forms and a temporary increase in the frequency of *Chilomastix*. In one instance, encystment occurred in the chick cecum, 4 days after the inoculation of trophozoites. The process of encystment in the chick, however, offers the same difficulty in determining the causes which bring it about, as it does in the normal host.

## Alkalinity of Gastric Venous Blood During Gastric Secretion.

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This study was undertaken to determine whether or not gastric HCl formation is accompanied by the simultaneous formation of an equivalent amount of alkali by the gastric tissue. The available alkali of gastric venous blood was compared with that of arterial blood simultaneously drawn during various conditions of gastric activity. The difference in available alkali in the 2 bloods is equal to the difference in bicarbonate ( $\Delta \text{BHCO}_3 = \text{BHCO}_3$  in gastric blood —  $\text{BHCO}_3$  in arterial blood) plus the difference in alkali bound by protein ( $\Delta \text{BP}$ ) at constant pH. The  $\text{BHCO}_3$  of the blood was calculated from Hasselbalch's equation,  $\text{pH} = \text{pK}^1 + \log \frac{\text{BHCO}_3}{\text{H}_2\text{CO}_3}$ , by substituting the values for pH and total  $\text{CO}_2$  experimentally determined. In calculating the difference in base bound by proteins of the 2 bloods 3 proteins must be considered, proteins of serum, Ps, hemoglobin, Hb, and oxyhemoglobin  $\text{HbO}_2$ .  $\Delta \text{BP}_{\text{blood}} = \Delta \text{BPs} + \Delta \text{BHbO}_2 + \Delta \text{BHb}$ . The following equations were used for calculating each of these.  $\Delta \text{pH}$  in any case is pH of gastric blood — pH of arterial blood.

$$\Delta \text{BPs} = 3.4 \times \Delta \text{pH serum.}$$

$$\Delta \text{BHb} = 3.35 \times \text{mmols Hb} \times \Delta \text{pH cells.}$$

$$\Delta \text{BHbO}_2 = 3.60 \times \text{mmols HbO}_2 \times \Delta \text{pH cells.}$$

Because of their relatively small capacity for combining with base, the proteins of serum were not determined but were assumed to be constant, and the equation  $\frac{d\text{BPs}}{d \text{pH}} = 3.4$  was assumed for all the samples of blood worked with. The pH of the cells, used in calculation of  $\Delta \text{BHb}$ , and  $\Delta \text{BHbO}_2$  was obtained from the pH of the serum, using the figure of Van Slyke, Wu and McLean,<sup>1</sup> giving the relation of cell and serum pH's. The values for  $\frac{d \text{BHbO}_2}{d \text{pH}}$ , and  $\frac{d \text{BHb}}{d \text{pH}}$ , are also those of Van Slyke, Wu, and McLean.<sup>2</sup> Oxyhemoglobin was obtained from oxygen content, and total hemoglobin from oxygen capacity determinations. The experimental determinations on the blood included pH of serum (by quinhydrone electrode),<sup>3</sup>  $\text{CO}_2$  con-

<sup>1</sup> Van Slyke, Wu, and McLean, *J. Biol. Chem.*, 1923, **56**, 778.

<sup>2</sup> Van Slyke, Wu, and McLean, *J. Biol. Chem.*, 1923, **56**, 811.

<sup>3</sup> Hanke, Martin E., *Proc. Soc. Exp. Biol. and Med.*, 1930, **27**, 972.

tent, oxygen content, and oxygen capacity. The blood gas determinations were made in the manometric gas apparatus of Van Slyke and Neill.<sup>4</sup>

Control determinations were first made to determine with what accuracy the addition of known amounts of HCl or NaOH to blood would be reflected in changes in their  $\text{BHCO}_3$  and BPr content. It was found that the addition of 3 or 4 millimols of HCl to normal dog or beef blood equilibrated with 40 mm.  $\text{CO}_2$  plus air to one atmosphere, was quantitatively reflected in a decrease of  $\text{BHCO}_3 + \text{BPr}$  content with a maximum error of 0.3 millimol.

For the gastric secretion experiments large dogs under barbital anesthesia were used. Histamine, one or 2 mg. per dog, given intramuscularly, was used as gastric stimulant, which usually resulted after one hour in marked gastric secretion, 50 cc. per hour containing N/10 HCl being a typical response. The serous side of the stomach was exposed through an incision in the abdominal wall, and the gastric venous blood was collected from one of the gastric veins attached to the outside of the serous membrane. The arterial blood was drawn from the femoral artery as soon as possible, within 10 minutes, after the gastric blood was drawn. For the determination of pH,  $\text{O}_2$  and  $\text{CO}_2$  content, the blood was drawn in tubes under oil, to protect against changes in gas content. Gastric juice was collected by stomach tube at noted time intervals, its volume measured, and its acidity determined by titration.

TABLE I.  
Arterial and Gastric Venous Blood.

Gastric Juice cc. per hour free acid	33 80		54 74		0 0	
	A <sub>2</sub>	G <sub>2</sub>	A <sub>3</sub>	G <sub>3</sub>	A <sub>1</sub>	G <sub>1</sub>
pH serum	7.19	7.26	7.27	7.36	7.40	7.43
mmols $\text{CO}_2$ blood	20.18	19.57	19.76	19.68	23.26	22.26
mmols $\text{HbO}_2$	8.53	8.72	8.04	7.55	8.22	9.84
mmols Hb	1.20	1.20	1.48	2.03	2.74	1.18
$\text{BHCO}_3$	18.53	18.16	18.40	18.56	22.15	21.27
$\Delta \text{BHCO}_3$ (G-A)		-0.37		+0.16		-0.88
$\Delta \text{BPr}$ (G-A)		+2.26		+2.69		+0.89
$\Delta \text{BHCO}_3 + \Delta \text{BPr}$ (G-A)		+1.89		+2.85		+0.01

The table gives the result of a typical experiment. When there was no gastric secretion, the difference between arterial and gastric venous blood, in the base bound by bicarbonate and protein, was

<sup>4</sup> Van Slyke and Neill, *J. Biol. Chem.*, 1924, **61**, 523.

found in 3 experiments to be at most 0.6 millimol. During active gastric secretion this difference in 4 experiments was from 1.8 to 3.5 millimols. The magnitude of the difference was roughly proportional to the rate of gastric acid secretion.

These results show that gastric acid formation is accompanied by the simultaneous formation of an approximately equivalent amount of alkali by the gastric tissue.

## Western New York Section.

*University of Rochester, February 28, 1931.*

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### Diet and the Blood Lipids.

W. R. BLOOR.

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The lipids, cholesterol and its fatty acid esters, phospholipids and fat are always found in the blood of animals. In the corpuscles, the content of cholesterol and phospholipid is about the same for all animals, in the plasma the content of these substances is variable, being in general much higher in the plasma of carnivora than in that of herbivora. It was at first thought that the plasma content of lipids was fairly constant and characteristic for each species and that it was possible to speak of normal levels and normal variations from these levels. Evidence is, however, accumulating which goes to show that these conceptions will have to be modified since there are often wide variations in individuals of the same species under similar conditions and still greater variations when the conditions are different. The greatest single factor influencing the level of the blood lipids appears to be diet and in particular the amount of fat which the food contains. Attention was called to this factor by results obtained by Glusker in this laboratory with dogs on a diet containing very little fat. Values for phospholipid were obtained far below any previously found in this laboratory or reported in the literature. Cholesterol values were also low but as markedly so as those of the phospholipids. The present report has to do with further feeding experiments on 4 of these dogs and on 4 rabbits. The dogs were alternated between the dog biscuit diet which contained 2.56% fat, 17.91% protein and 61.8% carbohydrates and a diet containing the same biscuit but with about 1/3 the caloric value replaced by lard. Variations of from about 140 mgm. % on the low fat to 190 mgm. % for phospholipid on the high fat diet and from about 65 mgm. % to 90 mgm. % for cholesterol were obtained. The very low phos-

pholipid values found in these dogs at the beginning of the present series of experiments (60 mgm. %) were not reached.

The rabbits were alternated between a nearly fat-free diet of hay, alfalfa and cabbage and a diet of sun-flower seeds, the meats of which contained about 40% of fat. On these diets the phospholipid values in the plasma differed very widely, the cholesterol less widely but definitely. Table I will illustrate the average results obtained on the first of the rabbits. Results on the others were similar.

TABLE I.

	Phospholipid mgm. %	Cholesterol mgm. %	Fat mgm. %	Total Lipid mgm. %
Low Fat Diet	25	18	100	130
High Fat Diet	175	70	150	370

The feeding periods were about one month long and both dogs and rabbits remained in good health and spirits, gaining weight slowly. The differences noted above were repeated 3 or more times on the same animals.

The results show that in the rabbits the level of the plasma lipids was closely dependent on the amount of fat in the food. The results with the dogs showed that the level of the plasma lipids was influenced by the fat in the diet but the effect was much less marked than in the rabbits and indicated that other factors were influential.

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### Increased Resistance to Cold Produced by Cortin After Adrenalectomy.\*

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It is well known that adrenalectomized animals resist cold poorly. This appears to be due to cortical rather than medullary deficiency, because cortical transplants enable adrenalectomized rats to resist cold well.<sup>1</sup> In the present study we have been able to show that an extract of the adrenal cortex protects adrenalectomized rats so that they resist cold very well.

\* Aided by a grant from the National Research Council.

<sup>1</sup> Wyman, L. C., and Tum Suden, C., *Am. J. Physiol.*, 1929, **89**, 362.

Two series of rats were completely adrenalectomized at one operation. The rats in series A (20 rats) were adrenalectomized 10 to 25 days before the experiment with cold. These were protected by injection of cortical extract twice daily (the product from 15 to 25 gm. of cortex each time). The rats in series B (19 rats) were adrenalectomized 4 to 25 days before the experiment. These were injected with the same volume of isotonic NaCl solution at the same time that the cortical extract was given to those in series A.

One extra injection of 0.5 cc. of the respective solutions was administered 12 hours before starting the test with cold, and others as follows: 4 hours before (0.5 cc.), at the test (0.4 cc.), and 4 hours after starting the test (0.5 cc.).

The rats had lived all their lives at a temperature of 27°C. The rats of series A and B together with 6 normal rats of the same weights were exposed to a temperature which gradually fell from 13°C. to 8°C. in 10 hours, when they were again placed in the warm room (27°C.).

The average changes in rectal temperature are shown in the accompanying table.

TABLE I.  
Rectal Temperatures of Rats Exposed to Cold.

	Start	6 hours	10 hours	14 hours
Normals	38.6°C.	37.0°C.	36.88°C.	38.45°C.
Cortical treated	37.76	37.14	35.33	37.33
NaCl treated	37.24	33.19	27.28	37.46

Four of the adrenalectomized, NaCl treated rats eventually died. None of the cortin treated animals fared the worse for their experience.

The cortical hormone, therefore, bears an important relation to the maintenance of body temperature.

